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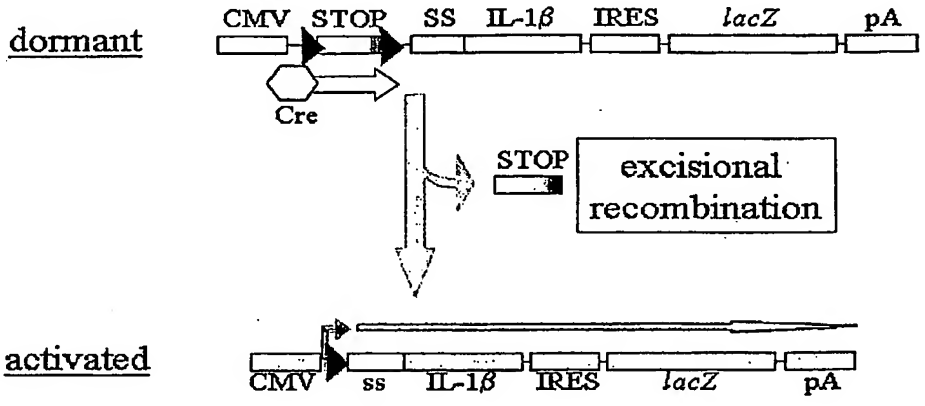
(54) Title: COMPOSITIONS AND METHODS FOR STUDYING AND TREATING INFLAMMATORY DISEASES AND DISORDERS



(57) Abstract: Disclosed are compositions and methods that can be used in the treatment of diseases and disorders caused, exacerbated or otherwise affected by inflammation.

WO 2006/079068 A2

COMPOSITIONS AND METHODS FOR STUDYING AND TREATING INFLAMMATORY DISEASES AND DISORDERS

I. CROSS-REFERENCE TO RELATED APPLICATIONS

1. This application claims benefit of U.S. Provisional Application No. 60/646,099 filed January 20, 2005, which is hereby incorporated herein by reference in its entirety.

II. BACKGROUND

2. There are a number of diseases and disorders related to inflammation, as well as a number of pathways and molecules related to inflammation. Disclosed are methods of treating inflammatory disease using compositions and methods identified herein.

III. SUMMARY

3. In accordance with the purposes of this invention, as embodied and broadly described herein, this invention, in one aspect, relates to vector constructs that can be used to inhibit inflammation and treat subjects with inflammatory disease.

4. Disclosed are methods and compositions related to polypeptides, nucleic acids, vectors, cells, transgenic animals for the study and treatment of inflammatory diseases and disorders, and methods of making and using thereof.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

5. The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments and together with the description illustrate the disclosed compositions and methods.

6. Figure 1 shows IL-1β^{XAT} – an excisionally activated transgene. IL-1β^{XAT} is a bicistronic gene comprized of the cytomegalovirus promoter (CMV), followed by a "floxed" transcriptional termination cassette (►STOP►), the human IL-1 RA peptide secretion signal (ss) fused to the mature human IL-1β ORF (ssIL-1β), the reporter lacZ gene and the bovine growth hormone poly A mRNA tail (pA). An internal ribosomal entry signal facilitates translation and expression of the second ORF, lacZ, at approximately 45% of the first ORF.

7. Figure 2 shows Cre-mediated activation of the inducible IL-1β^{XAT} transgene. The IL-1β^{XAT} gene was transfected into the murine fibroblast NIH 3T3 cell line. Transient expression of Cre recombinase following co-transfection of the expression vector pRc/CMV-CreWT resulted in IL-1β^{XAT} activation and higher levels of IL-1β mRNA detected by RT-PCR, as well as lacZ expression assessed by X-gal histochemistry (10X). Control conditions included (a) plain NIH 3T3 cells, as well as (b) cells co-transfected with IL-1β^{XAT} and (c) the pRc/CMV- backbone

vector, which displayed background levels of IL-1β and lacZ expression presumably due to minimal spontaneous read-through from the strong CMV promoter.

8. Figure 3 shows CrePr induces loxP-directed IL-1β^{XAT} excisional recombination and gene activation. The IL-1β^{XAT} gene was transiently transfected into 293HGLVP/CrePr cells and the expression of IL-1β and lacZ were evaluated following RU486 (10-7 M) administration. (A) Activation of Cre recombinase by RU486 resulted in up-regulation of both IL-1β and lacZ mRNA as assessed by RT-PCR. For demonstration purposes, an IL-1β standard curve (1µg -10-5 µg) is included in this panel. (B) Concomitantly, significantly higher levels of secreted IL-1β protein were found in the supernatant media of RU486-treated cells as assessed by ELISA for human IL-1β. (C) The expression of the reporter gene β-galactosidase was also confirmed by Xgal histochemistry: naïve cells present only minimal levels of background staining, whereas addition of RU486 in the culture media resulted in significant increase in the number of X-gal positive cells. (D) IL-1β^{XAT} excisional DNA recombination was confirmed by PCR of genomic DNA extracts from cells treated with plain growth media as well as media containing RU486 (10-7M) using a primer set (UP & LP) that flanked the ►STOP► sequence. PCR amplification of cells under plain media yielded a full-length product (~3Kb), indicative of a dormant IL-1β^{XAT} state. In contrast, RU486-treated cells yielded a PCR product of 1Kb in size, indicative of DNA recombination and excision of the ►STOP► cassette.

9. Figure 4 shows IL-1β^{XAT} activation results in expression of biologically potent IL-1β cytokine. The biological potency of the transgene–derived IL-1β cytokine was evaluated in vitro as follows. Murine fibroblasts were treated with conditioned media collected from cultured NIH 3T3 cells that had been previously transfected with Cre-induced IL-1β^{XAT} as described in Figure 2 above (co-transfection with the pRc/CMV-creWT vector). COX-2 transcript levels were measured as previously described in the target cells (murine fibroblasts) and was employed as a measure of IL-1β biological potency. Conditioned media were incubated with the neutralizing antibodies for 2 hours at 37°C prior to addition to target cells. (A) Conditioned medium collected form naïve NIH 3T3 cells (containing <3.9 pg/mL hIL-1β as determined by ELISA) were placed on murine fibroblasts, which in turn showed low levels of murine COX-2 mRNA. Moreover, (B) conditioned medium from NIH 3T3 cells transfected with IL-1β^{XAT} + pRc/CMV-backbone vector (contained <3.9 pg/mL hIL-1β) also showed low levels of murine COX-2 mRNA. In contrast, (C) conditioned medium from IL-1β^{XAT} + pRc/CMV- CreWT transfected NIH 3T3 cells (1 ng/mL hIL-1β) significantly induced COX-2 mRNA in the target cells; (D) pre-incubation of the conditioned medium with a control rabbit IgG antibody (5 µg/mL IgG1

isotype) had minimal effects on COX-2 regulation. However, (E) pre-incubation of the conditioned medium with a rabbit anti- hIL-1β (5 µg/mL IgG1) antibody attenuated the COX-2 induction. (F) Positive control: additional of human recombinant IL-1β (1 ng/mL+ 5 µg IgG1 isotype). (G) human recombinant IL-1β pre-incubated with 5 µg/mL neutralizing antibody. Results are shown as fold induction of COX-2 mRNA relative to group A. In conclusion, this experiment demonstrated that activation of the IL-1β^{XAT} gene results in production of biologically potent IL-1β and subsequently up-regulation of the inducible COX-2. (N=3). *$p<0.05$; S.E.M.

10. Figure 5 shows IL-1β induces inflammation-related genes. The effects of IL-1β were evaluated in vitro utilizing primary rat endothelial cell cultures as a representative rodent cell type. In this experiment, murine IL-1β (10ng/mL) was administered to cultured primary cells, and subsequently examined for the regulation of several inflammation-related genes at the transcript level over the course of 72 hours. These molecules include (A) the inducible isoform of cyclooxygenase (COX-2), intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1), as well as (B) the collagenase-A (MMP-2) and –B (MMP-9). Panel (C) depicts enzyme activity levels of MMP-2 and MMP-9 as evaluated by zymography.

11. Figure 6 shows Cre-mediated activation of the COLL1- IL1β^{XAT} gene. The 3.6 Kb promoter of the A1 chain of pro-collagen I gene, which has been shown to target gene expression in bone and cartilage, drove the expression of the IL-1β^{XAT} gene in NIH 3T3 stable cell line following trasfection with the pRc/CMV-CreWT vector and infection with the HIV(Cre) virus. Panel (A) depicts transfection (+) of such a stable cell line with pRc/CMV-CreWT, leading to expression of human IL-1β expression concomitantly with Cre recombinase as detected by RT-PCR. In contrast, untreated cells (-) were characterized by the absence of IL-1β and Cre recombinase. Panel (B) depicts similar IL-1β and Cre expression as assessed by RT-PCR following infection of the COLL1-IL1β^{XAT} cell line with the HIV(Cre) virus. The presence of IL-1β^{XAT} in the cells was confirmed by PCR as shown.

12. Figure 7 shows COLL1A1-IL-1β^{XAT} transgenic mice. Two series of micro-injections yielded 3 strong candidate transgenic COLL1A1-IL-1β^{XAT} mouse lines: #4, #11 and #12. This figure depicts PCR amplification of the transgene using a set of primers that also amplify the endogenous murine IL-1β gene at low levels. Transgene transmission was examined in the offspring of #4, 11 and 12 transgenic founders. F2 mice were bilaterally injected with FIV(cre) into the knee joint and are being monitored weekly for changes in locomotive behavior and

mass. c=control; 4, 11, 12, 13, 14 = Transgenic mouse lines; + = PCR positive control; - = PCR primers control.

13. Figure 8 shows mRNA knockdown by siRNA. siRNA knockdown of mRNA's encoding genes of the Prostaglandin E2 inflammatory pathway. NIH3T3's were transfected under standard condition with 100-200 nM chemically synthesized siRNA species and total RNA was collected 60 hrs later. mRNA knockdown was determined using QRT-PCR. Western immunoblotting was used to demonstrate siRNA knockdown of cPGES, mPGES, COX-2, and COX-1 protein. NIH3T3 cells were transfected with 200 nM of chemically synthesized siRNA complementary to the cPGES, mPGES, COX-2, and COX-1 , and total protein collected 60 hrs later. Samples were probed with an anti-mouse cPGES, mPGES, COX-2, and COX-1 antibody, respectively.

14. Figure 9 shows behavioral changes in Col1-IL1β^{XAT} mice after injection of FIV(Cre) in the knees. A group of Col1-IL1β^{XAT} transgenic mice (N=3) received a single intra-articular injection of 10^6 infectious particles of FIV(Cre) in the right and left knees at 2 months of age. In addition, a second group of mice (N=3) received saline injection and served as controls. During a session, each mouse was videotaped for 1 hour. The tape was then transferred digitally to a computer and analyzed in 20 periods of 3 minutes each. The duration of each mouse displaying grooming and licking was recorded and summed as seconds. The analysis of the behaviors was made by an investigator who was blind to the animal group assignment. Statistical analysis was performed by t-Test. Error bars = SEM. *=$P<0.05$.

15. Figure 10 shows locomotive deterioration in Col1-IL1β^{XAT} mice after injection of FIV(Cre) in the knees. Four groups of mice (N=3) were evaluated in terms of locomotive behavior by the rotorod appliance (Columbus Instruments, Columbus OH) and the lapse time until the mice well off the rotating cylinder (20 rpm) was recorded. The mice were evaluated over a period of 8 weeks following the intra-articular injections (8 wks – 16 wks of age).

16. Figure 11 shows FIV(Cre) injection in the knee of Col1-IL1β^{XAT} mice resulted in transgene induction. Immunocytochemical detection of the reporter gene β-galactosidase was employed to confirm the activation of the Col1-IL1β^{XAT} transgene by FIV(Cre) in this mouse model using antibodies raised against β-galactosidase and Cre recombinase. (A) FICT-conjugated immu-nodetection of β-galactosidase, (B) Texas Red-conjugated immunodetection of Cre recombinase, and (C) B/W image of the same microscopic field. (D) Overlap of panels A+B, and (E) overlap of panels A+B+C demonstrating co-expression of β-galactosidase and Cre

recombinase in vivo (solid arrows). All images were captured at a magnification of 20X. "m"=meniscus; "a"=articular surface; "I"=intra-articular space.

17. Figure 12 shows arthritic changes in the knee joint of Col1-IL1β^{XAT} mice following injection of FIV(Cre). (A) H&E staining of a knee section harvested from a 4 month old Col1-IL1β^{XAT} transgenic mouse injected with FIV(Cre) revealed the formation of fibrillations (solid arrow) and of an articular lip (open arrow). In contrast, (B) a transgenic mouse that received the control vector FIV(GFP) did not develop such anatomic aberrations. (C) Alcian blue / orange semi-quantitative evaluation showed a decrease in cartilage and bone density in the Col1-IL1β^{XAT}+FIV(Cre) knees compared to (D) controls. Moreover, increased cloning along with thickening of the articular surfaces was observed in the experimental animals (indicated by small arrows).

18. Figure 13 shows brain inflammation in Col1-IL1β^{XAT} mice following injection of FIV(Cre) in the knee and TMJ. Eight weeks after FIV(Cre) injection in the knee and TMJ of Col1-IL1β^{XAT} mice the brain was evaluated for activation of microglia and astrocytes by immunocytochemistry. (A) Using a monoclonal antibody raised against the MHC-class II antigen, the presence of activated microglia was detected in the brain. In contrast, control animals did not display any MHC-II positive cells. (C) Larger magnification of panel A. (B) There was lack of astrocyte activation in the brains of these animals as assessed by glial fibrillary acidic protein (GFAP). (D) Larger magnification of panel B.

19. Figure 14 shows arthritis-like changes in the TMJ of Col1-IL1β^{XAT} mice after intra-articular injection of FIV(Cre). Eight weeks after FIV(Cre) injection in the TMJ of Col1-IL1β^{XAT} mice anatomic aberrations of the joint were evaluated by semi-quantitative Alcian blue – orange G histochemistry. (A) TMJ section from an inactive Col1-IL1β^{XAT} mouse depicting the condylar head as well as the meniscus. In comparison, (B) a TMJ section harvested from a Col1-IL1β^{XAT} mouse injected with FIV(Cre) in the TMJ. (C) Larger magnification of the identified area of panel A. (D) Larger magnification of the identified area of panel B.

20. Figure 15 shows the inflammatory cascade and strategic targets in the cascade for gene and protein therapies. Figure 13, shows inhibition of IL-1β's biologic activity by a neutralizing antibody. (A) Conditioned medium collected form naïve NIH 3T3 cells were placed on murine fibroblasts, which in turn showed low levels of murine COX-2 mRNA. (B) Conditioned medium from NIH 3T3 cells transfected with IL-1β^{XAT} + pRc/CMV- backbone vector also showed low levels of murine COX-2 mRNA. In contrast, (C) conditioned medium from IL-1β^{XAT} + pRc/CMV-CreWT transfected NIH 3T3 cells significantly induced COX-2

mRNA in the target cells; (D) preincubation of the conditioned medium with a control rabbit IgG antibody (5 ng/mL IgG1 isotype) had minimal effects on COX-2 regulation. However, (E) pre-incubation of the conditioned medium with a rabbit anti- IL1β (5 ng/mL IgG1) antibody attenuated the COX-2 induction. (F) Positive control: addition of human recombinant IL-1β (1 ng/mL+ 5 ng IgG1 isotype). (G) human recombinant IL-1β pre-incubated with 5 ng/mL neutralizing antibody. Results are shown as fold induction of COX-2 mRNA relative to group A.

21. Figure 16 shows inhibition of IL-1β's biologic activity by a neutralizing antibody. (A) Conditioned medium collected form naïve NIH 3T3 cells were placed on murine fibroblasts, which in turn showed low levels of murine COX-2 mRNA. (B) Conditioned medium from NIH 3T3 cells transfected with IL-1β^{XAT} + pRc/CMV- backbone vector also showed low levels of murine COX-2 mRNA. In contrast, (C) conditioned medium from IL-1β^{XAT} + pRc/CMV-CreWT transfected NIH 3T3 cells significantly induced COX-2 mRNA in the target cells; (D) preincubation of the conditioned medium with a.control rabbit IgG antibody (5 ng/mL IgG1 isotype) had minimal effects on COX-2 regulation. However, (E) pre-incubation of the conditioned medium with a rabbit anti- IL1β (5 ng/mL IgG1) antibody attenuated the COX-2 induction. (F) Positive control: addition of human recombinant IL-1β (1 ng/mL+ 5 ng IgG1 isotype). (G) human recombinant IL-1β pre-incubated with 5 ng/mL neutralizing antibody. Results are shown as fold induction of COX-2 mRNA relative to group A.

22. Figure 17 shows FIV(IL1ra) successfully transduces cells with a gene expressing IL-1ra receptor antagonist. FIV(IL1ra) was constructed as depicted in panel A and confirmed by restriction enzyme analysis depicted in panel B. FIV(IL1ra) was then tested in vitro; IL1ra expression was evaluated in murine NIH 3T3 cells infected with this virus at the mRNA and protein levels. (C) RT-PCR analysis of infected cells demonstrated the expression of IL1ra mRNA. In contrast, naïve cells did not display any IL1ra expression. The housekeeping gene G3PDH was also employed. (D) IL1ra protein level in the media of injected cells was assessed by ELISA. Infection of cells by FIV(IL1ra) resulted in therapeutic IL1ra levels (>30 μg/mL). In contrast, FIV(gfp) and naïve cells did not express IL1ra.

V. DETAILED DESCRIPTION

23. The disclosed method and compositions can be understood more readily by reference to the following detailed description of particular embodiments and the Example included therein and to the Figures and their previous and following description.

24. Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that they are not limited to specific synthetic

methods or specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

A. Definitions

25. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

26. Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value "10" is disclosed the "less than or equal to 10"as well as "greater than or equal to 10" is also disclosed. It is also understood that the throughout the application, data is provided in a number of different formats, and that this data, represents endpoints and starting points, and ranges for any combination of the data points. For example, if a particular data point "10" and a particular data point 15 are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15.

27. In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

28. "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

29. "Primers" are a subset of probes which are capable of supporting some type of enzymatic manipulation and which can hybridize with a target nucleic acid such that the enzymatic manipulation can occur. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art which do not interfere with the enzymatic manipulation.

30. "Probes" are molecules capable of interacting with a target nucleic acid, typically in a sequence specific manner, for example through hybridization. The hybridization of nucleic acids is well understood in the art and discussed herein. Typically a probe can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art.

31. Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

B. Compositions and Methods

32. Disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds can not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular vector is disclosed and discussed and a number of vector components including the promoters are discussed, each and every combination and permutation of promoters and other vector components and the modifications that are possible unless specifically indicated to the contraryThus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these

additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

1. Inflammatory Disease

33. Provided herein are compositions, including polypeptides, nucleic acids, vectors, and cells, that can be used in the study and treatment of inflammatory diseases and disorders. Inflammation is a localized protective reaction of tissue to irritation, injury, or infection, characterized by pain, redness, swelling, and sometimes loss of function. As used herein, "inflammatory disorder" or "inflammatory disease" refers to any condition, disease or disorder wherein inflammation is involved, such as the sustained or chronic inflammation that occurs when tissues are injured by viruses, bacteria, trauma, chemicals, heat, cold or any other harmful stimulus. Irritation or discomfort can result from inflammation in a mammal due to, for example, skin inflammation, eye inflammation, gut inflammation or the like. Further, it is generally believed that chronic inflammation can increase the risk to develop other disease or ailment, such as osteoarthritis, autoimmune disease, cancer or the like.

34. In one aspect, the provided compositions and methods relate to the study and treatment of arthritis. Arthritis as a disease can include many different disorders and symptoms and can affect many parts of the body. Arthritis typically causes pain, loss of movement and sometimes swelling. Arthritis is actually a term used for a set of more than 100 current medical conditions. Arthritis is most commonly associated with older individuals, but can start as early as infancy. Some forms affect people in their young-adult years. A common aspect among arthritic conditions is that they affect the musculoskeletal system and specifically the joints - where two or more bones meet. Arthritis-related joint problems can include pain, stiffness, inflammation and damage to joint cartilage (the tough, smooth tissue that covers the ends of the bones, enabling them to glide against one another) and surrounding structures. Such damage can lead to joint weakness, instability and visible deformities depending on the location of joint involvement. Many of the arthritic conditions are systemic, in that they affect the whole body. In these diseases, arthritis can cause damage to virtually any bodily organ or system, including the heart, lungs, kidneys, blood vessels and skin.

35. Some different types of arthritis are Osteoarthritis, Rheumatoid arthritis, Gout, Ankylosing spondylitis, Juvenile arthritis, Systemic lupus erythematosus (lupus), Scleroderma, and Fibromyalgia. Osteoarthritis is a degenerative joint disease in which the cartilage that covers the ends of bones in the joint deteriorates, causing pain and loss of movement as bone begins to rub against bone. It is the most prevalent form of arthritis. Rheumatoid arthritis is an

autoimmune disease in which the joint lining becomes inflamed as part of the body's immune system activity. Rheumatoid arthritis is one of the most serious and disabling types, affecting mostly women. Gout affects mostly men. It is usually the result of a defect in body chemistry. This painful condition most often attacks small joints, especially the big toe. Fortunately, gout almost always can be completely controlled with medication and changes in diet. Ankylosing spondylitis is a type of arthritis that affects the spine. As a result of inflammation, the bones of the spine grow together. Juvenile arthritis is a general term for all types of arthritis that occur in children. Children can develop juvenile rheumatoid arthritis or childhood forms of lupus, ankylosing spondylitis or other types of arthritis. Systemic lupus erythematosus (lupus) is a disorder that can inflame and damage joints and other connective tissues throughout the body. Scleroderma is a disease of the body's connective tissue that causes a thickening and hardening of the skin. Fibromyalgia is a disorder in which widespread pain affects the muscles and attachments to the bone. It affects mostly women.

36. In another aspect, the provided compositions and methods relate to the study and treatment of neuroinflammation. Neuroinflammation, characterized by activated microglia and astrocytes and local expression of a wide range of inflammatory mediators, is a fundamental reaction to brain injury, whether by trauma, stroke, infection, or neurodegeneration. This local tissue response is surely part of a repair and restorative process. Yet, like many inflammatory conditions in peripheral diseases, neuroinflammation can contribute to the pathophysiology of CNS disorders. For example, in Alzheimer's disease (AD), glial-driven inflammatory responses to Aβ deposition are thought to promote neurodegeneration, as evidenced by the extent of neuroinflammation in AD, increased risk for AD with certain polymorphisms of proinflammatory cytokine genes, and reduction in disease risk for individuals taking nonsteroidal anti-inflammatory drugs (NSAIDs).

37. Considered herein is the use of the provided compositions and methods relate to the study and treatment of any inflammatory disease. Thus, the provided compositions and methods relate to the study and treatment of inflammatory bowel disease. The provided compositions and methods relate to the study and treatment of chronic dermatological disorders.

38. A particular advantage of the provided compositions and methods is the herein described ability to deliver inflammatory mediators, and the disclosed modulators thereof, to the brain by means of peripheral administration. For example, FIV vectors are disclosed herein that can deliver the herein disclosed nucleic acids to target sites within the subject. The disclosed FIV constructs can be delivered systemically by injection into the circulation or locally by injection

into the target site, such that either method of administration can result in the delivery of the nucleic acid to cells in the brain, such as, for example, microglia or astrocytes. The use of FIV vectors to deliver nucleic acids or transgenes to the brain following systemic administration is described in Patent Cooperation Treaty Application No.PCT/US03/13672 and U.S. Provisional Patent Application No.10/781,142, which are herein incorporated by reference in their entirety as they related to this teaching. Thus, neural inflammatory disorders, as disclosed herein, can be treated through delivery of an inflammatory mediator, as discussed herein, via, for example, injection in the joint of the subject. In addition, inflammatory conditions related to bone and/or joints can be treated by injection into the joint or through system injection or IP injection as discussed herein.

2. Inflammatory mediator

39. Inflammatory diseases such as arthritis and neuroinflammation can be treated in part by inhibiting the expression or activity of an inflammatory mediator. An inflammatory mediator, as used herein, refers to a protein that modulates inflammation and includes, for example, cytokines (e.g., IL-1β) prostaglandins (e.g., prostaglandin E_2 (PGE_2)), prostaglandin synthases (e.g., COX-1, COX-2, cPGES, and mPGES), and modulators thereof.

a) Interleukin-1

40. An example of an inflammatory mediator is interleukin-1 (IL-1). IL-1 is a potent immunomodulating cytokine that exists as two principal isoforms, IL-1α and IL-1β. These two molecules show significant divergence in sequence and have somewhat different roles with IL-1α generally thought to be involved in direct cell:cell communication, whereas IL-1β is secreted. Nevertheless, these two molecules act through the same membrane-associated receptor known as IL-1 receptor type 1 (IL-1R1) to promote a proinflammatory signaling cascade that includes the activation of NFκB and MAP kinases [Rothwell, N.J. and G.N. Luheshi. Trends Neurosci. (2000) 23:618-625].

41. At least two molecules have been identified that antagonize the effects of IL-1. IL-1 receptor antagonist (IL-1ra) competes for receptor binding, and IL-1 receptor type 2 (IL-1R2), which lacks an intracellular domain, is thought to serve as a decoy receptor [Rothwell, N.J. and G.N. Luheshi. Trends Neurosci. (2000) 23:618-625]. Expression of each of these molecules is regulated. The contribution of IL-1 to an inflammatory response therefore depends on the relative balance of expression between these family members [Arend, W.P. Cytokine & Growth Factor Rev. (2002) 13:323-340]. In one example, the mature form of IL-1β is attached to the

secretion signal from IL-1ra, which is the same sequence as the secretion signal sequence of IL-1β.

42. Lavage and explant studies from normal and osteoarthritic cartilage support the contention that cytokines are up regulated in disease states. Specifically, Moos et al. [Moos V, et al. (1999) J Rheumatol 26:870-9] have demonstrated that cartilage from knee or hip joints in 10 patients with osteoarthritis (OA) compared to controls demonstrated cytokines, including IL-1β that are up regulated in OA cartilage. Shin et al. [Shin S-j, et al. (2003) J Appl Physiol.; 95:308-13] examined the influence of mechanical stress on matrix turnover in the meniscus in the presence of IL-1β to determine the role of nitric oxide (NO) in these processes. Stimulation of proteoglycan release in response to compression was dependent on NOS2 regardless of the presence of IL-1. These finding suggest that IL-1 can modulate the effects of mechanical stress on extracellular matrix turnover through a pathway that is dependent on NO. Joosten et al. [Joosten LA, et al (1999) J Immunol; 163:5049-55] have demonstrated that blocking of IL-1 is a cartilage and bone protective therapy in destructive arthritis, whereas the TNF-alpha antagonist has little effect on tissue destruction. Webb et al. [Webb GR, et al. (1998) Osteoarthr & Cartil 6167-76] demonstrated that OA synovium supernatants contained higher concentrations of interleukin-1 beta (IL-1 beta) and interleukin-6 (IL-6) than normal synovial supernatants and neutralizing antibodies to these cytokines either partially or totally abrogated the ability of the OA supernatants to increase chondrocyte p55 TNF-R expression. These results suggest that IL-1 and IL-6 produced by OA synovium contribute to the progression of the disease by rendering chondrocytes more susceptible to stimulation by catabolic cytokines. Smith et al. [Smith MD, et al. (1997) J Rheumatol 24:365-71] examined the production of IL-1α, IL-1β and TNFα in synovial membranes from patients with OA, irrespective of the degree of articular cartilage damage. They suggest that chronic inflammatory changes with production of proinflammatory cytokines are a feature of synovial membranes from patients with early OA, with the most severe changes seen in patients at the time of joint replacement surgery. This low grade synovitis results in the production of cytokines that can contribute to the pathogenesis of OA.

43. Although both isoforms of IL-1 are made in brain, most work has focused on the role of IL-1β. Principally produced by microglia, IL-1β is rapidly induced following CNS injury. IL-1β affects many cellular targets, including astrocytes, neurons, and endothelial cells. In these cells, IL-1 up-regulates cytokines and chemokines, induces the expression of cell surface adhesion molecules and matrix metalloproteases, and stimulates cell proliferation [St Pierre, B.A., *et al.* Effects of cytokines on CNS cells: glia, in: (Ed.) Ransohoff, R.M., E.N. Beveniste,

Cytokines and the CNS, CRC Press, Boca Raton, (1996) pp. 151-168]. Moreover, it has been demonstrated that IL-1β induces COX-2 in brain astrocytes, leading to production of the proinflammatory prostaglandin PGE_2 [O'Banion, M.K., *et al.* Neurochem. (1996) 66:2532-2540]. Taken together, the myriad effects of IL-1 on multiple brain cell types suggest a critical role for IL-1 family members in coordinating brain neuroinflammatory responses.

44. The profound influence of IL-1 on neuroinflammation and its ubiquitous expression in conditions ranging from frank brain trauma to neurodegenerative disease suggests that it might contribute to CNS injury [Rothwell, N.J. and G.N. Luheshi. Trends Neurosci. (2000) 23:618-625]. This appears to be the case. For example, IL-1β is induced in experimental models of stroke [Minami, M., K. *et al.* J. Neurochem. (1992) 58:390-392] and infusion of IL-1β exacerbates damage whereas treatment with IL-1ra or IL-1 blocking antibodies significantly attenuates tissue injury [Loddick, S.A. and N.J. Rothwell. J. Cereb. Blood Flow Metab. (1996) 16:932-940 and Yamasaki, Y., N. Matsuura, H. Shozuhara, H. Onodera, Y. Itoyama and K. Kogure. Stroke (1995) 26:676-681]. Similarly, ischemic injury is significantly attenuated in interleukin-1 converting enzyme deficient mice [Friedlander, R.M., *et al.* J. Exp. Med. (1997) 185:933-940]. As another example, GFAP directed expression of a human IL-1ra transgene attenuates edema, cytokine production and neurological deficits in a murine model of closed head injury [Tehranian, R., S. *et al.* J. Neurotrauma (2002) 19:939-951]. Finally, studies of penetrating brain injury in mice lacking the type 1 IL-1 receptor showed dramatic attenuation in microglial activation, leukocyte infiltration, and astrocyte activation [Basu, A., *et al.* J. Neurosci. (2002) 22:6071-6082]. Expression of numerous inflammatory mediators, including vascular cell adhesion molecule-1, several cytokines, and COX-2 was also greatly reduced in the IL-1R1 knockout mice, indicating that the IL-1 signaling pathway is essential for glial activation and the neuroinflammatory response. However, short-term infusion and viral delivery systems do not provide chronic stimuli and the genetic knockout systems are complicated by potential compensatory changes during development.

b) cyclooxygenase COX

45. Another example of an inflammatory mediator is the enzyme cyclooxygenase (COX). Cyclooxygenase is the principal target of non-steroidal anti-inflammatory drugs (NSAIDs), which are a mainstay of treatment for many inflammatory conditions. Cyclooxygenase catalyzes the first step in the conversion of arachidonic acid to prostanoids, a group of potent lipid mediators acting in diverse physiological processes.

46. Cyclooxygenase is known to exist in two isoforms: COX-1, which in many tissues appears to be constitutively expressed and responsible for homeostatic production of prostanoids, and COX-2, which is often referred to as the "inducible" isoform since its expression is rapidly modulated in response to diverse stimuli such as growth factors, cytokines, and hormones [O'Banion MK, et al. (1991). J Biol Chem 266: 23261-7; O'Banion MK, et al. (1992). Proc Natl Acad Sci U.S.A. 89:4888-92]. The distinction between these two COX isoforms, the roles they play, and the actions of prostanoids have been previously reviewed [Vane JR, et al. (1998). Annu. Rev. Pharmocol. Toxicol. 38:97-120; Smith, WL, et al. (2000). Annu Rev Biochem 69:145-82].

47. Interest in selectively inhibiting production of PGE_2, the principle inflammatory prostanoid, has been heightened by recognition of at least two PGE_2 synthase isoforms that are reportedly coupled to distinct COX isoforms. More specifically, a membrane-associated isoform (mPGES) is functionally coupled to COX-2, whereas a cytosolic enzyme (cPGES) appears to be linked to a COX-1-dependent production of PGE_2 (Tanioka et al. 2000; Murakami et al., 2000). Although cellular localization can play some role, functional coupling is largely a factor of expression patterns: as with COX-2, mPGES is dramatically upregulated by proinflammatory stimuli, whereas cPGES is constitutively expressed in cell systems examined to date (Jackobson et al., 1999; Stichtenoth et al., 2001; Han et al., 2002). In addition, COX-2 and mPGES are coordinately upregulated in a rat model of adjuvant arthritis (Mancini et al., 2001).

3. Inhibition

48. Provided herein are compositions that act to modulate an activity of an inflammatory mediator. "Activity," as used herein, refers to any function or process of a composition disclosed herein and includes, for example, transcription, translation, post-translational modification, translocation, homophilic or heterophilic binding, secretion, endocytosis, or degredation. Disclosed therefore are compositions that inhibit one or more activities of an inflammatory mediator provided herein. These compositions are refered to herein as inflammatory mediator inhibitors. Inhibition or a form of inhibition, such as inhibit or inhibiting, as used herein means to restrain or limit. Reduce or a form of reduce, such as reducing or reduces, as used herein, means to diminish, for example in size or amount. It is understood that wherever one of inhibit or reduce is used, unless explicitly indicated otherwise, the other can also be used. For example, if something is referred to as "inhibited," it is also considered referred to as "reduced."

a) knockdown of gene expression

49. The activity of an inflammatory mediator can be modulated at the gene expression level. The disclosed inflammatory mediator inhibitor can be a gene expression inhibitor. Methods of targeting gene expression are generally based on the sequence of the gene to be targeted. Disclosed are any such methods that can be applied to the targeted knockdown of an inflammatory mediator. By "knockdown" is meant a decrease in detectable mRNA expression. Nucleic acids are generally used to knockdown gene expression and generally comprise a sequence capable of hybridizing to the target sequence, such as mRNA. Examples of such functional nucleic acids include antisense molecules, ribozymes, triplex forming nucleic acids, external guide sequences (EGS), and small interfering RNAs (siRNA).

50. Antisense molecules are designed to interact with a target nucleic acid molecules through either canonical or non-canonical base pairing. The interaction of the antisense molecule and the target molecule is designed to promote the destruction of the target molecule through, for example, RNAseH mediated RNA-DNA hybrid degradation. Alternatively the antisense molecule is designed to interrupt a processing function that normally would take place on the target molecule, such as transcription or replication. Antisense molecules can be designed based on the sequence of the target molecule. Numerous methods for optimization of antisense efficiency by finding the most accessible regions of the target molecule exist. Exemplary methods would be in vitro selection experiments and DNA modification studies using DMS and DEPC. It is preferred that antisense molecules bind the target molecule with a dissociation constant (k_d) less than or equal to 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. A representative sample of methods and techniques which aid in the design and use of antisense molecules can be found in the following non-limiting list of United States patents: 5,135,917, 5,294,533, 5,627,158, 5,641,754, 5,691,317, 5,780,607, 5,786,138, 5,849,903, 5,856,103, 5,919,772, 5,955,590, 5,990,088, 5,994,320, 5,998,602, 6,005,095, 6,007,995, 6,013,522, 6,017,898, 6,018,042, 6,025,198, 6,033,910, 6,040,296, 6,046,004, 6,046,319, and 6,057,437. However, the effect of iRNA or siRNA or their use is not limited to any type of mechanism.

51. Disclosed herein are any antisense molecules designed as described above based on the sequences for the herein disclosed inflammatory mediators. Examples of antisense sequences are disclosed herein for IL-1α (SEQ ID NO:70), IL-1β (SEQ ID NO:71), COX-1 (SEQ ID NO:72), COX-2 (SEQ ID NO:73), cPGES (SEQ ID NO:74), and mPGES (SEQ ID NO:75).

52. Ribozymes are nucleic acid molecules that are capable of catalyzing a chemical reaction, either intramolecularly or intermolecularly. Ribozymes are thus catalytic nucleic acid.

It is preferred that the ribozymes catalyze intermolecular reactions. There are a number of different types of ribozymes that catalyze nuclease or nucleic acid polymerase type reactions which are based on ribozymes found in natural systems, such as hammerhead ribozymes, (for example, but not limited to the following United States patents: 5,334,711, 5,436,330, 5,616,466, 5,633,133, 5,646,020, 5,652,094, 5,712,384, 5,770,715, 5,856,463, 5,861,288, 5,891,683, 5,891,684, 5,985,621, 5,989,908, 5,998,193, 5,998,203, WO 9858058 by Ludwig and Sproat, WO 9858057 by Ludwig and Sproat, and WO 9718312 by Ludwig and Sproat) hairpin ribozymes (for example, but not limited to the following United States patents: 5,631,115, 5,646,031, 5,683,902, 5,712,384, 5,856,188, 5,866,701, 5,869,339, and 6,022,962), and tetrahymena ribozymes (for example, but not limited to the following United States patents: 5,595,873 and 5,652,107). There are also a number of ribozymes that are not found in natural systems, but which have been engineered to catalyze specific reactions de novo (for example, but not limited to the following United States patents: 5,580,967, 5,688,670, 5,807,718, and 5,910,408). Preferred ribozymes cleave RNA or DNA substrates, and more preferably cleave RNA substrates. Ribozymes typically cleave nucleic acid substrates through recognition and binding of the target substrate with subsequent cleavage. This recognition is often based mostly on canonical or non-canonical base pair interactions. This property makes ribozymes particularly good candidates for target specific cleavage of nucleic acids because recognition of the target substrate is based on the target substrates sequence. Representative examples of how to make and use ribozymes to catalyze a variety of different reactions can be found in the following non-limiting list of United States patents: 5,646,042, 5,693,535, 5,731,295, 5,811,300, 5,837,855, 5,869,253, 5,877,021, 5,877,022, 5,972,699, 5,972,704, 5,989,906, and 6,017,756.

53. Disclosed herein are any ribozymes designed as described above based on the sequences for the herein disclosed inflammatory mediators. Hammerhead ribozymes can cleave RNA substrates at for example, a 5'-GUC-3' sequence, cleaving the mRNA immediately 3' to the GUC site. Engineered hammerhead ribozymes, which cleave at a different sequence are known and disclosed, for example, in the patents disclosed herein, and are incorporated by reference. A hammerhead ribozyme is typically composed of three parts. The first part is a region which will hybridize to the sequence 5' of the GUC recognition site, and can be called a first hybridzation arm. A second part consists of a core catalytic domain of the hammerhead ribozyme, and typically has the sequence $^{3'}$CAAAGCAGGAGUGCCUGAGUAGUC$^{5'}$ (SEQ ID NO:82). Variations on this sequence are known and are herein disclosed and incorporated by reference, for example, in the patents disclosed herein. A third part consists of sequence capable

or hybridizing to the sequence immediately 3' to the GUC cleavage site, and can be called a second hybridization arm. The hybiridization arms can be any lebght allowing binding to the substrate, such as, from 3-40 nucleotides long, 5-30 nucleotides long, 8-20, nucleotides long and 10-15 nucleotides long, as well as any length up to 50 nucleotides. As an example, hammerhead ribozymes can be designed by identifying a nucleic acid triplet GUC within the mRNA target sequence, and then identifying the approproiate hybridizing arms as discussed herein to the catalytic core as discussed herein. Examples of hammerhead ribozyme sequences are disclosed herein for IL-1α (SEQ ID NO:76), IL-1β (SEQ ID NO:77), COX-1 (SEQ ID NO:78), COX-2 (SEQ ID NO:79), cPGES (SEQ ID NO:81), and mPGES (SEQ ID NO:80), but it is understood that others are also disclosed as discussed herein. Furthermore, using assays as discussed herein, one can test a given ribozyme (or any functional nucleic acid, such as an siRNA or antisense) for its level of activity, both in vitro and in vivo.

54. Triplex forming functional nucleic acid molecules are molecules that can interact with either double-stranded or single-stranded nucleic acid. When triplex molecules interact with a target region, a structure called a triplex is formed, in which there are three strands of DNA forming a complex dependant on both Watson-Crick and Hoogsteen base-pairing. Triplex molecules are preferred because they can bind target regions with high affinity and specificity. It is preferred that the triplex forming molecules bind the target molecule with a k_d less than 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. Representative examples of how to make and use triplex forming molecules to bind a variety of different target molecules can be found in the following non-limiting list of United States patents: 5,176,996, 5,645,985, 5,650,316, 5,683,874, 5,693,773, 5,834,185, 5,869,246, 5,874,566, and 5,962,426.

55. External guide sequences (EGSs) are molecules that bind a target nucleic acid molecule forming a complex, and this complex is recognized by RNase P, which cleaves the target molecule. EGSs can be designed to specifically target a RNA molecule of choice. RNAse P aids in processing transfer RNA (tRNA) within a cell. Bacterial RNAse P can be recruited to cleave virtually any RNA sequence by using an EGS that causes the target RNA:EGS complex to mimic the natural tRNA substrate. (WO 92/03566 by Yale, and Forster and Altman, Science 238:407-409 (1990)). Similarly, eukaryotic EGS/RNAse P-directed cleavage of RNA can be utilized to cleave desired targets within eukarotic cells. (Yuan et al., Proc. Natl. Acad. Sci. USA 89:8006-8010 (1992); WO 93/22434 by Yale; WO 95/24489 by Yale; Yuan and Altman, EMBO J 14:159-168 (1995), and Carrara et al., Proc. Natl. Acad. Sci. (USA) 92:2627-2631 (1995)). Representative examples of how to make and use EGS molecules to facilitate cleavage of a

variety of different target molecules are found in the following non-limiting list of United States patents: 5,168,053, 5,624,824, 5,683,873, 5,728,521, 5,869,248, and 5,877,162.

56. Gene expression can also be effectively silenced in a highly specific manner through RNA interference (RNAi). This silencing was originally observed with the addition of double stranded RNA (dsRNA) (Fire,A., et al. (1998) *Nature*, 391, 806 811) (Napoli, C., et al. (1990) *Plant Cell* 2, 279 289) (Hannon, G.J. (2002) *Nature*, 418, 244 251). Once dsRNA enters a cell, it is cleaved by an RNase III –like enzyme, Dicer, into double stranded small interfering RNAs (siRNA) 21-23 nucleotides in length that contains 2 nucleotide overhangs on the 3' ends (Elbashir, S.M., et al. (2001) *Genes Dev.*, 15:188-200) (Bernstein, E., et al. (2001) *Nature*, 409, 363 366) (Hammond, S.M., et al. (2000) *Nature*, 404:293-296). In an ATP dependent step, the siRNAs become integrated into a multi-subunit protein complex, commonly known as the RNAi induced silencing complex (RISC), which guides the siRNAs to the target RNA sequence (Nykanen, A., et al. (2001) *Cell*, 107:309 321). At some point the siRNA duplex unwinds, and it appears that the antisense strand remains bound to RISC and directs degradation of the complementary mRNA sequence by a combination of endo and exonucleases (Martinez, J., et al. (2002) *Cell*, 110:563-574). However, the effect of iRNA or siRNA or their use is not limited to anytype of mechanism.

57. Short Interfering RNA (siRNA) is a double-stranded RNA that can induce sequence-specific post-transcriptional gene silencing, thereby decreasing or even inhibiting gene expression. In one example, an siRNA triggers the specific degradation of homologous RNA molecules, such as mRNAs, within the region of sequence identity between both the siRNA and the target RNA. For example, WO 02/44321 discloses siRNAs capable of sequence-specific degradation of target mRNAs when base-paired with 3' overhanging ends, herein incorporated by reference for the method of making these siRNAs. Sequence specific gene silencing can be achieved in mammalian cells using synthetic, short double-stranded RNAs that mimic the siRNAs produced by the enzyme dicer (Elbashir, S.M., et al. (2001) *Nature*, 411:494 498) (Ui-Tei, K., et al. (2000) *FEBS Lett* 479:79-82). siRNA can be chemically or *in vitro*-synthesized or can be the result of short double-stranded hairpin-like RNAs (shRNAs) that are processed into siRNAs inside the cell. Synthetic siRNAs are generally designed using algorithms and a conventional DNA/RNA synthesizer. Suppliers include Ambion (Austin, Texas), ChemGenes (Ashland, Massachusetts), Dharmacon (Lafayette, Colorado), Glen Research (Sterling, Virginia), MWB Biotech (Esbersberg, Germany), Proligo (Boulder, Colorado), and Qiagen (Vento, The Netherlands). siRNA can also be synthesized *in vitro* using kits such as Ambion's *SILENCER*

siRNA Construction Kit. Disclosed herein are any siRNA designed as described above based on the sequences for the herein disclosed inflammatory mediators. Examples of siRNA include: COX-1 (SEQ ID NOs:47-52), COX-2 (SEQ ID NOs:53-58), cPGES (SEQ ID NOs:41-46), and mPGES (SEQ ID NO:59).

58. The production of siRNA from a vector is more commonly done through the transcription of a shRNA. Kits for the production of vectors comprising shRNA are available, such as for example Imgenex's GeneSuppressor Construction Kits and Invitrogen's BLOCK-iT inducible RNAi plasmid and lentivirus vectors. Disclosed herein are any shRNA designed as described above based on the sequences for the herein disclosed inflammatory mediators. Examples of shRNA primer sequences are disclosed for COX-1 (SEQ ID NOs:64-65), COX-2 (SEQ ID NOs:66-67), cPGES (SEQ ID NOs:60-61), and mPGES (SEQ ID NO:62-63).

b) Inhibition of Binding

59. Another activity of an inflammatory mediator that can be targeted is homophilic and heterophilic binding to other molecules, such as, for example, receptors. Thus, the inflammatory mediator inhibitor can be a ligand binding inhibitor. Methods for inhibiting the binding of a protein to its receptor can, for example, be based on the use of molecules that compete for the binding site of either the ligand or the receptor.

60. Thus, a ligand binding inhibitor can be, for example, a polypeptide that competes for the binding of a receptor without activating the receptor. Likewise, a ligand binding inhibitor can be a decoy receptor that competes for the binding of ligand. Such a decoy receptor can be a soluble receptor (e.g., lacking transmembrane domain) or it can be a mutant receptor expressed in a cell but lacking the ability to transduce a signal (e.g., lacking cytoplasmic tail). Antibodies specific for either a ligand or a receptor can also be used to inhibit binding. The ligand binding inhibitor can also be naturally produced by a subject. Alternatively, the inhibitory molecule can be designed based on targeted mutations of either the receptor or the ligand.

61. Thus, as an illustrative example, the ligand binding inhibitor can be IL-1 receptor antagonist (IL-1ra). The ligand binding inhibitor can also be a polypeptide comprising a fragment of IL-1ra, wherein the fragment is capable of binding IL-1R1. ligand binding inhibitor can further be IL-1R2, which is a soluble form of the receptor that can compete for IL-1 binding. The ligand binding inhibitor can further be a polypeptide comprising a fragment of IL-1R1. The IL-1R1 fragment can lack the cytoplasmic tail, which includes the Toll/interleukin-1(IL-1) receptor (TIR) domain (amino acids 384-528 of SEQ ID NO:8). The fragment of IL-1R1 can lack the amino acids corresponding to the transmembrane domain.

4. Antibodies

Antibodies specific for inflammatory mediators or their receptors can be used herein. For example, disclosed for use in the provided compositions and methods are neutralizing antibodies specific for IL-1β or IL-1 receptor, or nucleic acids encoding said antibodes.

(1) Antibodies Generally

The term "antibodies" is used herein in a broad sense and includes both polyclonal and monoclonal antibodies. In addition to intact immunoglobulin molecules, also included in the term "antibodies" are fragments or polymers of those immunoglobulin molecules, and human or humanized versions of immunoglobulin molecules or fragments thereof, as long as they are chosen for their ability to interact with inflammatory mediators or their receptors such that inflammatory mediators is inhibited from interacting with its receptor. The antibodies can be tested for their desired activity using the *in vitro* assays described herein, or by analogous methods, after which their *in vivo* therapeutic and/or prophylactic activities are tested according to known clinical testing methods.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a substantially homogeneous population of antibodies, i.e., the individual antibodies within the population are identical except for possible naturally occurring mutations that may be present in a small subset of the antibody molecules. The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, as long as they exhibit the desired antagonistic activity (See, U.S. Pat. No. 4,816,567 and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

The disclosed monoclonal antibodies can be made using any procedure which produces mono clonal antibodies. For example, disclosed monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized in vitro, e.g., using the HIV Env-CD4-co-receptor complexes described herein.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567 (Cabilly et al.). DNA encoding the disclosed monoclonal antibodies can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Libraries of antibodies or active antibody fragments can also be generated and screened using phage display techniques, e.g., as described in U.S. Patent No. 5,804,440 to Burton et al. and U.S. Patent No. 6,096,441 to Barbas et al.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. For instance, digestion can be performed using papain. Examples of papain digestion are described in WO 94/29348 published Dec. 22, 1994 and U.S. Pat. No. 4,342,566. Papain digestion of antibodies typically produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual Fc fragment. Pepsin treatment yields a fragment that has two antigen combining sites and is still capable of cross-linking antigen.

The fragments, whether attached to other sequences or not, can also include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the antibody or antibody fragment is not significantly altered or impaired compared to the non-modified antibody or antibody fragment. These modifications can provide for some additional property, such as to remove/add amino acids capable of disulfide bonding, to increase its bio-longevity, to alter its secretory characteristics, etc. In any case, the antibody or antibody fragment must possess a bioactive property, such as specific binding to its cognate antigen. Functional or active regions of the antibody or antibody fragment may be identified by mutagenesis of a specific region of the protein, followed by expression and testing of the expressed polypeptide. Such methods are readily apparent to a skilled practitioner in the art and can include site-specific mutagenesis of the nucleic acid encoding the antibody or antibody fragment. (Zoller, M.J. Curr. Opin. Biotechnol. 3:348-354, 1992).

As used herein, the term "antibody" or "antibodies" can also refer to a human antibody and/or a humanized antibody. Many non-human antibodies (e.g., those derived from mice, rats, or rabbits) are naturally antigenic in humans, and thus can give rise to undesirable immune responses when administered to humans. Therefore, the use of human or humanized antibodies

in the methods serves to lessen the chance that an antibody administered to a human will evoke an undesirable immune response.

(2) Human antibodies

The disclosed human antibodies can be prepared using any technique. Examples of techniques for human monoclonal antibody production include those described by Cole et al. (*Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77, 1985) and by Boerner et al. (J. Immunol., 147(1):86-95, 1991). Human antibodies (and fragments thereof) can also be produced using phage display libraries (Hoogenboom et al., J. Mol. Biol., 227:381, 1991; Marks et al., J. Mol. Biol., 222:581, 1991).

The disclosed human antibodies can also be obtained from transgenic animals. For example, transgenic, mutant mice that are capable of producing a full repertoire of human antibodies, in response to immunization, have been described (see, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551-255 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggermann et al., Year in Immunol., 7:33 (1993)). Specifically, the homozygous deletion of the antibody heavy chain joining region (J(H)) gene in these chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production, and the successful transfer of the human germ-line antibody gene array into such germ-line mutant mice results in the production of human antibodies upon antigen challenge. Antibodies having the desired activity are selected using Env-CD4-co-receptor complexes as described herein.

(3) Humanized antibodies

Antibody humanization techniques generally involve the use of recombinant DNA technology to manipulate the DNA sequence encoding one or more polypeptide chains of an antibody molecule. Accordingly, a humanized form of a non-human antibody (or a fragment thereof) is a chimeric antibody or antibody chain (or a fragment thereof, such as an Fv, Fab, Fab', or other antigen-binding portion of an antibody) which contains a portion of an antigen binding site from a non-human (donor) antibody integrated into the framework of a human (recipient) antibody.

To generate a humanized antibody, residues from one or more complementarity determining regions (CDRs) of a recipient (human) antibody molecule are replaced by residues from one or more CDRs of a donor (non-human) antibody molecule that is known to have desired antigen binding characteristics (e.g., a certain level of specificity and affinity for the target antigen). In some instances, Fv framework (FR) residues of the human antibody are replaced by corresponding non-human residues. Humanized antibodies may also contain

residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. Humanized antibodies generally contain at least a portion of an antibody constant region (Fc), typically that of a human antibody (Jones et al., Nature, 321:522-525 (1986), Reichmann et al., Nature, 332:323-327 (1988), and Presta, Curr. Opin. Struct. Biol., 2:593-596 (1992)).

Methods for humanizing non-human antibodies are well known in the art. For example, humanized antibodies can be generated according to the methods of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986), Riechmann et al., Nature, 332:323-327 (1988), Verhoeyen et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Methods that can be used to produce humanized antibodies are also described in U.S. Patent No. 4,816,567 (Cabilly et al.), U.S. Patent No. 5,565,332 (Hoogenboom et al.), U.S. Patent No. 5,721,367 (Kay et al.), U.S. Patent No. 5,837,243 (Deo et al.), U.S. Patent No. 5, 939,598 (Kucherlapati et al.), U.S. Patent No. 6,130,364 (Jakobovits et al.), and U.S. Patent No. 6,180,377 (Morgan et al.).

(4) Administration of antibodies

Administration of the antibodies can be done as disclosed herein. Nucleic acid approaches for antibody delivery also exist. The broadly neutralizing anti xxx antibodies and antibody fragments can also be administered to patients or subjects as a nucleic acid preparation (e.g., DNA or RNA) that encodes the antibody or antibody fragment, such that the patient's or subject's own cells take up the nucleic acid and produce and secrete the encoded antibody or antibody fragment. The delivery of the nucleic acid can be by any means, as disclosed herein, for example.

C. Compositions

62. Disclosed herein are constructs capable of inhibiting an activity of an inflammatory mediator. In one aspect, the constructs are vectors comprising a nuceic acid, wherein the nucleic acid encodes for the inhibitor. The nucleic acids of the constructs can be based on the sequence of an inflammatory mediator.

1. Inflammatory Mediators – Sequences

63. The disclosed constructs can comprise a nucleic acid based on the sequence of IL-1 alpha. The nucleic acid sequence can be based on the sequence of human IL-1 alpha. An

example of a nucleic acid encoding human IL-1 alpha is SEQ ID NO:1, Accession No. NM_000575.

64. The disclosed constructs can comprise a nucleic acid based on the sequence of IL-1 beta. The nucleic acid sequence can based on the sequence of human IL-1 beta. An example of a nucleic acid encoding human IL-1 beta is SEQ ID NO:2, Accession No. NM_000576.

65. The disclosed constructs can comprise a nucleic acid based on the sequence of IL-1ra. The nucleic acid sequence can based on the sequence of human IL-1ra. An example of a nucleic acid encoding human IL-1ra is SEQ ID NO:5, Accession No. NM_173842.

66. The disclosed constructs can comprise a nucleic acid based on the sequence of IL-1R1. The nucleic acid sequence can based on the sequence of human IL-1RA. An example of a nucleic acid encoding human IL-1R1 is SEQ ID NO:8, Accession No. NM_000877.

67. The disclosed constructs can comprise a nucleic acid based on the sequence of IL-1R2. The nucleic acid sequence can based on the sequence of human IL-1R2. An example of a nucleic acid encoding human IL-1R2 is SEQ ID NO:9, Accession No. NM_173343.

68. The disclosed constructs can comprise a nucleic acid based on the sequence of COX-1. The nucleic acid sequence can based on the sequence of human COX-1. An example of a nucleic acid encoding human COX-1 is SEQ ID NO:10, Accession No. M59979.

69. The disclosed constructs can comprise a nucleic acid based on the sequence of COX-2. The nucleic acid sequence can based on the sequence of human COX-2. An example of a nucleic acid encoding human COX-2 (SEQ ID NO:11, Accession No. NM_000963.

70. The disclosed constructs can comprise a nucleic acid based on the sequence of mPGES. The nucleic acid sequence can based on the sequence of human mPGES. An example of a nucleic acid encoding human mPGES is SEQ ID NO:12, Accession No. NM_004878.

71. The disclosed constructs can comprise a nucleic acid based on the sequence of cPGES. The nucleic acid sequence can based on the sequence of human cPGES/p23. An example of a nucleic acid encoding human cPGES/p23 is SEQ ID NO:13, Accession No. L24804.

72. Disclosed herein is a functional nucleic acid wherein the nucleic acid can inhibit the expression of a mediator of inflammation. Also disclosed herein is a construct comprising a nucleic acid encoding the functional nucleic acid operably linked to an expression control sequence. The functional nucleic acid can comprise an siRNA. The siRNA can be derived from the nucleic acid sequence for COX-1 (SEQ ID NO:10). Thus, the siRNA can have the nucleic acid sequence SEQ ID NO:47, 48, 49, 50, 51, or 52. The siRNA can be derived from the nucleic

acid sequence for COX-2 (SEQ ID NO:11). Thus, the siRNA can have the nucleic acid sequence SEQ ID NO:53, 54, 555, 56, 57, or 58. The siRNA can be derived from the nucleic acid sequence for mPGES (SEQ ID NO:12). Thus, the siRNA can have the nucleic acid sequence SEQ ID NO:59. The siRNA can be derived from the nucleic acid sequence for cPGES (SEQ ID NO:13). Thus, the siRNA can have the nucleic acid sequence SEQ ID NO:41,,42, 43, 44, 45, or 46.

73. Disclosed herein is a construct comprising a nucleic acid encoding a polypeptide operably linked to an expression control sequence, wherein the polypeptide can inhibit the binding of IL-1 to IL-1R1. The polypeptide can comprise IL-1ra. The polypeptide can have the amino acid sequence SEQ ID NO:38. The polypeptide can comprise a fragment of IL-1ra. The polypeptide can have at least 70%, 75%, 80%, 85%, 90%, 95% identity to the amino acid sequence SEQ ID NO:38. The nucleic acid can comprise the sequence SEQ ID NO:5. The nucleic acid encode a polypeptide that with at least 70%, 75%, 80%, 85%, 90%, 95% identity to the nucleic acid sequence SEQ ID NO:5. Also disclosed are nucleic acids that can hybridize under stringent conditions, or other conditions, as described herein, with the nucleic acid sequence SEQ ID NO:5.

74. The polypeptide can comprise a fragment of IL-1R1, wherein the fragment is capable of binding IL-1 and wherein the fragment has a reduced ability to activate a signal cascase. It is understood that one skilled in the art can readily determine the ability of a polypeptide to bind IL-1 or activate a signal cascase using standard biochemical and molecular genetics techniques and reagents. As an example, the fragment can be a truncation lacking the transmembrane domain. Wherein the transmembrane domain has not been identified, it is understood that one skilled in the art can estimate the approximate location of this domain based on the amino acid sequence using, for example, hydrophobicity plots. As another example, the fragment can lack part of the cytoplasmic tail, which includes the Toll/interleukin-1(IL-1) receptor (TIR) domain (amino acids 384-528 of SEQ ID NO:8). The polypeptide can have the amino acid sequence SEQ ID NO:39. The polypeptide can have at least 70%, 75%, 80%, 85%, 90%, 95% identity to the amino acid sequence SEQ ID NO:39. The nucleic acid can comprise the sequence SEQ ID NO:8. The nucleic acid encode a polypeptide that with at least 70%, 75%, 80%, 85%, 90%, 95% identity to the nucleic acid sequence SEQ ID NO:8. Also disclosed are nucleic acids that can hybridize under stringent conditions, or other conditions, as described herein, with the nucleic acid sequence SEQ ID NO:8.

75. The polypeptide can comprise IL-1R2. The polypeptide can have the amino acid sequence SEQ ID NO:40. The polypeptide can comprise a fragment of IL-1R2, wherein the fragment is capable of binding IL-1 and wherein the fragment has a reduced ability to activate a signal cascase. The polypeptide can have at least 70%, 75%, 80%, 85%, 90%, 95% identity to the amino acid sequence SEQ ID NO:40. The nucleic acid can comprise the sequence SEQ ID NO:9. The nucleic acid encode a polypeptide that with at least 70%, 75%, 80%, 85%, 90%, 95% identity to the nucleic acid sequence SEQ ID NO:9. Also disclosed are nucleic acids that can hybridize under stringent conditions, or other conditions, as described herein, with the nucleic acid sequence SEQ ID NO:9.

76. The herein disclosed polypeptides can further comprise a secretion signal. The secretion signal can be the IL-1ra secretion signal sequence, which is the same sequence as the secretion signal sequence of IL-1β. Thus, the secretion signal can comprise the polypeptide sequence SEQ ID NO:14. The secretion signal can be encoded by nucleic acid sequence SEQ ID NO:68.

77. The disclosed constructs can be integrated into a vector delivery system. Thus, disclosed are vectors comprising the nucleic acids provided herein. The expression control sequence is generally a promoter. The promoter can be any promoter, such as those discussed herein.

78. Targeted and global delivery of the constructs provided herein is also disclosed. Disclosed is a pseudotyped feline immunodeficiency virus (FIV) for global transgene delivery. Stable expression of the therapeutic gene aids prolonged restoration of the genetic anomaly enhancing treatment efficacy and contributing to long-term therapeutic outcomes. One of the backbone FIV systems disclosed herein is set forth in Poeschla EM, et al., Nature Medicine 4: 354-357. (1998). For example, disclosed herein is stable expression of the reporter gene *lacZ* for over 3 months in mice following perinatal systemic FIV(lacZ) administration.

79. A model system for the study of these constructs is the IL-1β exisionally activated transgenic (XAT) mouse (IL-1β^{XAT}) and variations thereof. Variations include the use of tissue specific promoters such as in for example the COLL1A1-IL-1β^{XAT} mouse. This mouse model is the subject of U.S. Patent Application No. 60/627,604, which is herein incorporated by reference in its entirety for teachings related to the disclosed mouse models. This mouse model allows for the induction of localized inflammation based on the delivery of a Cre recombinase expression vector such as FIV(Cre) to the target site. For example, the delivery of FIV(Cre) to the joints of the COLL1A1-IL-1β^{XAT} mouse can induce inflammation to model arthritis. This mouse model

can thus be used to, for example, test or optimize the effects of the provided constructs on arthritis. Also disclosed herein is the ability of FIV vectors to deliver any of the herein provided nucleic acids or transgenes to the brain of a subject following injection of the vector into either the circulation or joints. Thus, the IL-1β^{XAT} and variations thereof can be used as a model of neuroinflammation following delivery of FIV(Cre) into the circulation or joints.

2. Nucleic acids

80. There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example, IL-1ra as well as any other proteins disclosed herein, as well as various functional nucleic acids. The disclosed nucleic acids are made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell, that the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantagous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

a) Nucleotides and related molecules

81. A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. A non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

82. A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to nucleotides are well known in the art and would include for example, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties.

83. Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate

moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

84. It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety. (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989,86, 6553-6556),

85. A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

86. A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH2 or O) at the C6 position of purine nucleotides.

b) Sequences

87. There are a variety of sequences related to, for example, IL-1ra as well as any other protein disclosed herein that are disclosed on Genbank, and these sequences and others are herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

88. A variety of sequences are provided herein and these and others can be found in Genbank, at www.pubmed.gov. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences. Primers and/or probes can be designed for any sequence given the information disclosed herein and known in the art.

c) Primers and probes

89. Disclosed are compositions including primers and probes, which are capable of interacting with the genes disclosed herein. In certain embodiments the primers are used to support DNA amplification reactions. Typically the primers will be capable of being extended in a sequence specific manner. Extension of a primer in a sequence specific manner includes any methods wherein the sequence and/or composition of the nucleic acid molecule to which the primer is hybridized or otherwise associated directs or influences the composition or sequence of

the product produced by the extension of the primer. Extension of the primer in a sequence specific manner therefore includes, but is not limited to, PCR, DNA sequencing, DNA extension, DNA polymerization, RNA transcription, or reverse transcription. Techniques and conditions that amplify the primer in a sequence specific manner are preferred. In certain embodiments the primers are used for the DNA amplification reactions, such as PCR or direct sequencing. It is understood that in certain embodiments the primers can also be extended using non-enzymatic techniques, where for example, the nucleotides or oligonucleotides used to extend the primer are modified such that they will chemically react to extend the primer in a sequence specific manner. Typically the disclosed primers hybridize with the nucleic acid or region of the nucleic acid or they hybridize with the complement of the nucleic acid or complement of a region of the nucleic acid.

3. Sequence similarities

90. It is understood that as discussed herein the use of the terms homology and identity mean the same thing as similarity. Thus, for example, if the use of the word homology is used between two non-natural sequences it is understood that this is not necessarily indicating an evolutionary relationship between these two sequences, but rather is looking at the similarity or relatedness between their nucleic acid sequences. Many of the methods for determining homology between two evolutionarily related molecules are routinely applied to any two or more nucleic acids or proteins for the purpose of measuring sequence similarity regardless of whether they are evolutionarily related or not.

91. In general, it is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein, is through defining the variants and derivatives in terms of homology to specific known sequences. This identity of particular sequences disclosed herein is also discussed elsewhere herein. In general, variants of genes and proteins herein disclosed typically have at least, about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent homology to the stated sequence or the native sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

92. Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment

algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

93. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods can differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity, and be disclosed herein.

94. For example, as used herein, a sequence recited as having a particular percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using the Zuker calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

4. Hybridization/selective hybridization

95. The term hybridization typically means a sequence driven interaction between at least two nucleic acid molecules, such as a primer or a probe and a gene. Sequence driven interaction means an interaction that occurs between two nucleotides or nucleotide analogs or nucleotide

derivatives in a nucleotide specific manner. For example, G interacting with C or A interacting with T are sequence driven interactions. Typically sequence driven interactions occur on the Watson-Crick face or Hoogsteen face of the nucleotide. The hybridization of two nucleic acids is affected by a number of conditions and parameters known to those of skill in the art. For example, the salt concentrations, pH, and temperature of the reaction all affect whether two nucleic acid molecules will hybridize.

96. Parameters for selective hybridization between two nucleic acid molecules are well known to those of skill in the art. For example, in some embodiments selective hybridization conditions can be defined as stringent hybridization conditions. For example, stringency of hybridization is controlled by both temperature and salt concentration of either or both of the hybridization and washing steps. For example, the conditions of hybridization to achieve selective hybridization can involve hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-25°C below the Tm (the melting temperature at which half of the molecules dissociate from their hybridization partners) followed by washing at a combination of temperature and salt concentration chosen so that the washing temperature is about 5°C to 20°C below the Tm. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA hybridizations. The conditions can be used as described above to achieve stringency, or as is known in the art. (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. Methods Enzymol. 1987:154:367, 1987 which is herein incorporated by reference for material at least related to hybridization of nucleic acids). A preferable stringent hybridization condition for a DNA:DNA hybridization can be at about 68°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 68°C. Stringency of hybridization and washing, if desired, can be reduced accordingly as the degree of complementarity desired is decreased, and further, depending upon the G-C or A-T richness of any area wherein variability is searched for. Likewise, stringency of hybridization and washing, if desired, can be increased accordingly as homology desired is increased, and further, depending upon the G-C or A-T richness of any area wherein high homology is desired, all as known in the art.

97. Another way to define selective hybridization is by looking at the amount (percentage) of one of the nucleic acids bound to the other nucleic acid. For example, in some

embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the limiting nucleic acid is bound to the non-limiting nucleic acid. Typically, the non-limiting primer is in for example, 10 or 100 or 1000 fold excess. This type of assay can be performed at under conditions where both the limiting and non-limiting primer are for example, 10 fold or 100 fold or 1000 fold below their k_d, or where only one of the nucleic acid molecules is 10 fold or 100 fold or 1000 fold or where one or both nucleic acid molecules are above their k_d.

98. Another way to define selective hybridization is by looking at the percentage of primer that gets enzymatically manipulated under conditions where hybridization is required to promote the desired enzymatic manipulation. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer is enzymatically manipulated under conditions which promote the enzymatic manipulation, for example if the enzymatic manipulation is DNA extension, then selective hybridization conditions would be when at least about 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer molecules are extended. Preferred conditions also include those suggested by the manufacturer or indicated in the art as being appropriate for the enzyme performing the manipulation.

99. Just as with homology, it is understood that there are a variety of methods herein disclosed for determining the level of hybridization between two nucleic acid molecules. It is understood that these methods and conditions can provide different percentages of hybridization between two nucleic acid molecules, but unless otherwise indicated meeting the parameters of any of the methods would be sufficient. For example if 80% hybridization was required and as long as hybridization occurs within the required parameters in any one of these methods it is considered disclosed herein.

100. It is understood that those of skill in the art understand that if a composition or method meets any one of these criteria for determining hybridization either collectively or singly it is a composition or method that is disclosed herein.

5. Delivery of the compositions to cells

101. The herein disclosed nucleic acids can be delivered to cells or cells in a subject. There are a number of compositions and methods which can be used to deliver nucleic acids to

cells, either in vitro or in vivo. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based delivery systems. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991). Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modifed to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

a) Nucleic acid based delivery systems

Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. Cancer Res. 53:83-88, (1993)).

As used herein, plasmid or viral vectors are agents that transport the disclosed nucleic acids, such as, for example, the IL-1ra, COX-1 siRNA, COX-2 siRNA, cPGES siRNA, or mPGES siRNA constructs into the cell without degradation and include a promoter yielding expression of the disclosed sequences in the cells into which it is delivered. In some embodiments the vectors for the IL-1ra, COX-1 siRNA, COX-2 siRNA, cPGES siRNA, or mPGES siRNA constructs are derived from a virus, retrovirus, or lentivirus. Viral vectors can be, for example, Adenovirus, Adeno-associated virus, Herpes virus, Vaccinia virus, Polio virus, AIDS virus, neuronal trophic virus, Sindbis and other RNA viruses, including these viruses with the HIV backbone, and lentiviruses. Also preferred are any viral families which share the properties of these viruses which make them suitable for use as vectors. Retroviruses include Murine Maloney Leukemia virus, MMLV, and retroviruses that express the desirable properties of MMLV as a vector. Retroviral vectors are able to carry a larger genetic payload, i.e., a transgene, such as, the disclosed IL-1ra, COX-1 siRNA, COX-2 siRNA, cPGES siRNA, or mPGES siRNA constructs or marker gene, than other viral vectors, and for this reason are a commonly used vector. However, they are not as useful in non-proliferating cells. Adenovirus

vectors are relatively stable and easy to work with, have high titers, and can be delivered in aerosol formulation, and can transfect non-dividing cells. Pox viral vectors are large and have several sites for inserting genes, they are thermostable and can be stored at room temperature. A preferred embodiment is a viral vector, which has been engineered so as to suppress the immune response of the host organism, elicited by the viral antigens. Preferred vectors of this type will carry coding regions for Interleukin 8 or 10.

Viral vectors can have higher transaction (ability to introduce genes) abilities than chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA. Constructs of this type can carry up to about 8 kb of foreign genetic material. The necessary functions of the removed early genes are typically supplied by cell lines which have been engineered to express the gene products of the early genes in *trans*.

(1) Retroviral Vectors

A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In Microbiology-1985, American Society for Microbiology, pp. 229-232, Washington, (1985), which is incorporated by reference herein. Examples of methods for using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116 and 4,980,286; PCT applications WO 90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993)); the teachings of which are incorporated herein by reference.

A retrovirus is essentially a package which has packed into it nucleic acid cargo. The nucleic acid cargo carries with it a packaging signal, which ensures that the replicated daughter molecules will be efficiently packaged within the package coat. In addition to the package signal, there are a number of molecules which are needed in cis, for the replication, and packaging of the replicated virus. Typically a retroviral genome, contains the gag, pol, and env genes which are involved in the making of the protein coat. It is the gag, pol, and env genes which are typically replaced by the foreign DNA that it is to be transferred to the target cell.

Retrovirus vectors typically contain a packaging signal for incorporation into the package coat, a sequence which signals the start of the gag transcription unit, elements necessary for reverse transcription, including a primer binding site to bind the tRNA primer of reverse transcription, terminal repeat sequences that guide the switch of RNA strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serve as the priming site for the synthesis of the second strand of DNA synthesis, and specific sequences near the ends of the LTRs that enable the insertion of the DNA state of the retrovirus to insert into the host genome. The removal of the gag, pol, and env genes allows for about 8 kb of foreign sequence to be inserted into the viral genome, become reverse transcribed, and upon replication be packaged into a new retroviral particle. This amount of nucleic acid is sufficient for the delivery of a one to many genes depending on the size of each transcript. It is preferable to include either positive or negative selectable markers along with other genes in the insert.

Since the replication machinery and packaging proteins in most retroviral vectors have been removed (gag, pol, and env), the vectors are typically generated by placing them into a packaging cell line. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the helper cell. The genomes for the machinery are not packaged because they lack the necessary signals.

(2) Adenoviral Vectors

The construction of replication-defective adenoviruses has been described (Berkner et al., J. Virology 61:1213-1220 (1987); Massie et al., Mol. Cell. Biol. 6:2872-2883 (1986); Haj-Ahmad et al., J. Virology 57:267-274 (1986); Davidson et al., J. Virology 61:1226-1239 (1987); Zhang "Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis" BioTechniques 15:868-872 (1993)). The benefit of the use of these viruses as vectors is that they are limited in the extent to which they can spread to other cell types, since they can replicate within an initial infected cell, but are unable to form new infectious viral particles. Recombinant adenoviruses have been shown to achieve high efficiency gene transfer after direct, in vivo delivery to airway epithelium, hepatocytes, vascular endothelium, CNS parenchyma and a number of other tissue sites (Morsy, J. Clin. Invest. 92:1580-1586 (1993); Kirshenbaum, J. Clin. Invest. 92:381-387 (1993); Roessler, J. Clin.

Invest. 92:1085-1092 (1993); Moullier, Nature Genetics 4:154-159 (1993); La Salle, Science 259:988-990 (1993); Gomez-Foix, J. Biol. Chem. 267:25129-25134 (1992); Rich, Human Gene Therapy 4:461-476 (1993); Zabner, Nature Genetics 6:75-83 (1994); Guzman, Circulation Research 73:1201-1207 (1993); Bout, Human Gene Therapy 5:3-10 (1994); Zabner, Cell 75:207-216 (1993); Caillaud, Eur. J. Neuroscience 5:1287-1291 (1993); and Ragot, J. Gen. Virology 74:501-507 (1993)). Recombinant adenoviruses achieve gene transduction by binding to specific cell surface receptors, after which the virus is internalized by receptor-mediated endocytosis, in the same manner as wild type or replication-defective adenovirus (Chardonnet and Dales, Virology 40:462-477 (1970); Brown and Burlingham, J. Virology 12:386-396 (1973); Svensson and Persson, J. Virology 55:442-449 (1985); Seth, et al., J. Virol. 51:650-655 (1984); Seth, et al., Mol. Cell. Biol. 4:1528-1533 (1984); Varga et al., J. Virology 65:6061-6070 (1991); Wickham et al., Cell 73:309-319 (1993)).

A viral vector can be one based on an adenovirus which has had the E1 gene removed and these virons are generated in a cell line such as the human 293 cell line. In another preferred embodiment both the E1 and E3 genes are removed from the adenovirus genome.

(3) Adeno-asscociated viral vectors

Another type of viral vector is based on an adeno-associated virus (AAV). This defective parvovirus is a preferred vector because it can infect many cell types and is nonpathogenic to humans. AAV type vectors can transport about 4 to 5 kb and wild type AAV is known to stably insert into chromosome 19. Vectors which contain this site specific integration property are preferred. An especially preferred embodiment of this type of vector is the P4.1 C vector produced by Avigen, San Francisco, CA, which can contain the herpes simplex virus thymidine kinase gene, HSV-tk, and/or a marker gene, such as the gene encoding the green fluorescent protein, GFP.

In another type of AAV virus, the AAV contains a pair of inverted terminal repeats (ITRs) which flank at least one cassette containing a promoter which directs cell-specific expression operably linked to a heterologous gene. Heterologous in this context refers to any nucleotide sequence or gene which is not native to the AAV or B19 parvovirus.

Typically the AAV and B19 coding regions have been deleted, resulting in a safe, noncytotoxic vector. The AAV ITRs, or modifications thereof, confer infectivity and site-specific integration, but not cytotoxicity, and the promoter directs cell-specific expression.

United states Patent No. 6,261,834 is herein incorproated by reference for material related to the AAV vector.

The vectors of the present invention thus provide DNA molecules which are capable of integration into a mammalian chromosome without substantial toxicity.

The inserted genes in viral and retroviral usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and can contain upstream elements and response elements.

(4) Lentiviral vectors

102. The vectors can be lentiviral vectors, including but not limited to, SIV vectors, HIV vectors or a hybrid construct of these vectors, including viruses with the HIV backbone. These vectors also include first, second and third generation lentiviruses. Third generation lentiviruses have lentiviral packaging genes split into at least 3 independent plasmids or constructs. Also vectors can be any viral family that share the properties of these viruses which make them suitable for use as vectors. Lentiviral vectors are a special type of retroviral vector which are typically characterized by having a long incubation period for infection. Furthermore, lentiviral vectors can infect non-dividing cells. Lentiviral vectors are based on the nucleic acid backbone of a virus from the lentiviral family of viruses. Typically, a lentiviral vector contains the 5' and 3' LTR regions of a lentivirus, such as SIV and HIV. Lentiviral vectors also typically contain the Rev Responsive Element (RRE) of a lentivirus, such as SIV and HIV.

(a) Feline immunodeficiency viral vectors

One type of vector that the disclosed constructs can be delivered in is the VSV-G pseudotyped Feline Immunodeficiency Virus system developed by Poeschla *et al.* (1998). This lentivirus has been shown to efficiently infect dividing, growth arrested as well as post-mitotic cells. Furthermore, due to its lentiviral properties, it allows for incorporation of the transgene into the host's genome, leading to stable gene expression. This is a 3-vector system, whereby each confers distinct instructions: the FIV vector carries the transgene of interest and lentiviral apparatus with mutated packaging and envelope genes. A vesicular stomatitis virus G-glycoprotein vector (VSV-G; Burns et al., 1993) contributes to the formation of the viral envelope *in trans*. The third vector confers packaging instructions *in trans* (Poeschla *et al.*,

1998). FIV production is accomplished *in vitro* following co-transfection of the aforementioned vectors into 293-T cells. The FIV-rich supernatant is then collected, filtered and can be used directly or following concentration by centrifugation. Titers routinely range between $10^4 - 10^7$ bfu/ml..

(5) Packaging vectors

As discussed above, retroviral vectors are based on retroviruses which contain a number of different sequence elements that control things as diverse as integration of the virus, replication of the integrated virus, replication of un-integrated virus, cellular invasion, and packaging of the virus into infectious particles. While the vectors in theory could contain all of their necessary elements, as well as an exogenous gene element (if the exogenous gene element is small enough) typically many of the necessary elements are removed. Since all of the packaging and replication components have been removed from the typical retroviral, including lentiviral, vectors which will be used within a subject, the vectors need to be packaged into the initial infectious particle through the use of packaging vectors and packaging cell lines. Typically retroviral vectors have been engineered so that the myriad functions of the retrovirus are separated onto at least two vectors, a packaging vector and a delivery vector. This type of system then requires the presence of all of the vectors providing all of the elements in the same cell before an infectious particle can be produced. The packaging vector typically carries the structural and replication genes derived from the retrovirus, and the delivery vector is the vector that carries the exogenous gene element that is preferably expressed in the target cell. These types of systems can split the packaging functions of the packaging vector into multiple vectors, e.g., third-generation lentivirus systems. Dull, T. et al., "A Third-generation lentivirus vector with a conditional packaging system"J. Virol 72(11):8463-71 (1998)

Retroviruses typically contain an envelope protein (env). The Env protein is in essence the protein which surrounds the nucleic acid cargo. Furthermore cellular infection specificity is based on the particular Env protein associated with a typical retrovirus. In typical packaging vector/delivery vector systems, the Env protein is expressed from a separate vector than for example the protease (pro) or integrase (in) proteins.

(6) Packaging cell lines

The vectors are typically generated by placing them into a packaging cell line. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the

vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the helper cell. The genomes for the machinery are not packaged because they lack the necessary signals. One type of packaging cell line is a 293 cell line.

(7) Large payload viral vectors

Molecular genetic experiments with large human herpesviruses have provided a means whereby large heterologous DNA fragments can be cloned, propagated and established in cells permissive for infection with herpesviruses (Sun et al., Nature genetics 8: 33-41, 1994; Cotter and Robertson,.Curr Opin Mol Ther 5: 633-644, 1999). These large DNA viruses (herpes simplex virus (HSV) and Epstein-Barr virus (EBV), have the potential to deliver fragments of human heterologous DNA > 150 kb to specific cells. EBV recombinants can maintain large pieces of DNA in the infected B-cells as episomal DNA. Individual clones carried human genomic inserts up to 330 kb appeared genetically stable The maintenance of these episomes requires a specific EBV nuclear protein, EBNA1, constitutively expressed during infection with EBV. Additionally, these vectors can be used for transfection, where large amounts of protein can be generated transiently in vitro. Herpesvirus amplicon systems are also being used to package pieces of DNA > 220 kb and to infect cells that can stably maintain DNA as episomes.

Other useful systems include, for example, replicating and host-restricted non-replicating vaccinia virus vectors.

b) Non-nucleic acid based systems

103. The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example in vivo or in vitro.

104. Thus, in addition to the disclosed nucleic acids or vectors, the compositions can comprise, for example, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al. *Proc. Natl.*

Acad. Sci USA 84:7413-7417 (1987); U.S. Pat. No.4,897,355. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

105. In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the disclosed nucleic acid or vector can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

106. The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). These techniques can be used for a variety of other speciifc cell types. Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization

pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

107. Nucleic acids that are delivered to cells which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral related sequences, particularly when viral based systems are used. These viral intergration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

108. Other general techniques for integration into the host genome include, for example, systems designed to promote homologous recombination with the host genome. These systems typically rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid to be integrated into the host genome. These systems and the methods necessary to promote homologous recombination are known to those of skill in the art.

c) In vivo/ex vivo

109. As described above, the compositions can be administered in a pharmaceutically acceptable carrier and can be delivered to the subject's cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

110. If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

6. Expression systems

The nucleic acids that are delivered to cells typically contain expression controlling systems. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

a) Viral Promoters and Enhancers

Preferred promoters controlling transcription from vectors in mammalian host cells can be obtained from various sources, for example, the genomes of viruses such as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin promoter. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course, promoters from the host cell or related species also are useful herein.

Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl. Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol. Cell Bio. 3: 1108 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33: 729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4: 1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Preferred examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

The promoter and/or ennancer can be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating chemotherapy drugs.

In certain embodiments the promoter and/or enhancer region can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular type of cell at a particular time. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTF.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) can also contain sequences necessary for the termination of transcription which could affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. It is also preferred that the transcribed units contain other standard sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

111. In certain embodiments the promoters are constitutive promoters. This can be any promoter that causes transcription regulation in the absence of the addition of other factors. Examples of this type of promoter are the CMV promoter and the beta actin promoter, as well as others dicussed herein. In certain embodiments the promoter can consist of fusions of one or more different types of promoters. For example, the regulatory regions of the CMV promoter and the beta actin promoter are well known and understood, examples, of which are disclosed herein. Parts of these promoters can be fused together to, for example, produce a CMV-beta actin fusion promoter, such as the one shown in SEQ ID NO:18 . It is understood that this type of promoter has a CMV component and a beta actin component. These components can function

independently as promoters, and thus, are themselves considered beta actin promoters and CMV promoters. A promoter can be any portion of a known promoter that causes promoter activity. It is well understood that many promoters, including the CMV and Beta Actin promoters have functional domains which are understood and that these can be used as a beta actin promoter or CMV promoter. Furthermore, these domains can be determined. For example, SEQ ID NO:s 15-33 display a number of CMV promoters, beta actin promoters, and fusion promoters. These promoters can be compared, and for example, functional regions delineated, as described herein. Furthermore, each of these sequences can function independently or together in any combination to provide a promoter region for the disclosed nucleic acids.

112. The promoters can also be non-constitutive promoters, such as cell specific promoters. These are promoters that are turned on at specific time in development or stage or a particular type of cell, such as a cardiac cell, or neural cell, or a bone cell. Some examples of cell specific promoters are, the neural enolase specifc promoter (NSE), the procollagen promoters COL1A1 (SEQ ID NO:35) and COL2A1 (SEQ ID NO:36), the CD11b promoter (PBMC-microglia/macrophage/monocyte specific) (SEQ ID NO:69), and the glial specific glial fibrillary acetic protein (GFAP) promoter (SEQ ID NO:34).

113. It is understood that the recombinant systems can be expressed in a tissue-specific manner. It is understood that tissue specific expression can occur due to the presence of a tissue-specific promoter. Typically, proteins under control of a tissue-specific promoter are transcribed when the promoter becomes active by virtue of being present in the tissue for which it is specific. Therefore, all cells can encode for a particular gene without global expression. As such, labeled proteins can be shown to be present in certain tissues without expression in other nearby tissues that could complicate results or expression of proteins in tissues where expression is detrimental to the host. Disclosed are methods wherein the cre recombinase is under the control of the EIIA promoter, a promoter specific for breast tissue, such as the WAP promoter, a promoter specific for ovarian tissue, such as the ACTB promoter, or a promoter specific for bone tissue, such as osteocalcin. Any tissues specific promoter can be used. Promoters specific for prostate, testis, and neural are also disclosed. Examples of some tissue-specific promoters include but are not limited to MUC1, EIIA, ACTB, WAP, bHLH-EC2, HOXA-1, Alpha-fetoprotein (AFP), opsin, CR1/2, Fc-γ-Receptor 1 (Fc-γ-R1), MMTVD-LTR, the human insulin promote, Pdha-2. For example, use of the AFP promoter creates specificity for the liver. Another example, HOXA-1 is a neuronal tissue specific promoter, and as such, proteins expressed under the control of HOXA-

1 are only expressed in neuronal tissue. Sequences for these and other tissue-specific promoters are known in the art and can be found, for example, in Genbank, at www.pubmed.gov.

b) Markers

114. The viral vectors can include nucleic acid sequence encoding a marker product. This marker product is used to determine if the gene has been delivered to the cell and once delivered is being expressed. Preferred marker genes are the *E. Coli* lacZ gene, which encodes ß-galactosidase, and green fluorescent protein.

115. In some embodiments the marker can be a selectable marker. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hydromycin, and puromycin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are: CHO DHFR- cells and mouse LTK- cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

116. The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such dominant selection use the drugs neomycin, (Southern P. and Berg, P., J. Molec. Appl. Genet. 1: 327 (1982)), mycophenolic acid, (Mulligan, R.C. and Berg, P. Science 209: 1422 (1980)) or hygromycin, (Sugden, B. et al., Mol. Cell. Biol. 5: 410-413 (1985)). The three examples employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid) or hygromycin, respectively. Others include the neomycin analog G418 and puramycin.

c) Post transcriptional regulatory elements

117. The disclosed vectors can also contain post-transcriptional regulatory elements. Post-transcriptional regulatory elements can enhance mRNA stability or enhance translation of the transcribed mRNA. An exemplary post-transcriptional regulatory sequence is the WPRE sequence isolated from the woodchuck hepatitis virus. [Zufferey R, et al., J Virol; 73:2886-92 (1999)]. Post-transcriptional regulatory elements can be positioned both 3' and 5' to the exogenous gene, but it is preferred that they are positioned 3' to the exogenous gene.

d) Transduction efficiency elements

118. Transduction efficiency elements are sequences that enhance the packaging and transduction of the vector. These elements typically contain polypurine sequences. An example of a transduction efficiency element is the ppt-cts sequence that contains the central polypurine tract (ppt) and central terminal site (cts) from the HIV-1 pSG3 molecular clone (SEQ ID NO:1 bp 4327 to 4483 of HIV-1 pSG3 clone).

e) 3' untranslated regions

119. Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) may also contain sequences necessary for the termination of transcription which could affect mRNA expression. These 3' untranslated regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding the exogenous gene. The 3' untranslated regions also include transcription termination sites. The transcription unit also can contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. Homologous polyadenylation signals can be used in the transgene constructs. In an embodiment of the transcription unit, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. Transcribed units can contain other standard sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

7. Peptides

a) Protein variants

120. Disclosed herein are constructs comprising nucleic acids that encode polypeptides. As discussed herein, there can be numerous variants of each of these polypeptides, such as IL-1ra, that are herein contemplated. In addition, to the known functional proteins that are disclosed, such as IL-1ra, there are also derivatives of these proteins which also function in

the disclosed methods and compositions. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof can be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 1 and 2 and are referred to as conservative substitutions.

TABLE 1: Amino Acid Abbreviations

Amino Acid	Abbreviations	
Alanine	Ala	A
allosoleucine	Alle	
Arginine	Arg	R
asparagine	Asn	N
aspartic acid	Asp	D
Cysteine	Cys	C
glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isolelucine	Ile	I

Leucine	Leu	L
Lysine	Lys	K
phenylalanine	Phe	F
proline	Pro	P
pyroglutamic acid	pGlu	
Serine	Ser	S
Threonine	Thr	T
Tyrosine	Tyr	Y
Tryptophan	Trp	W
Valine	Val	V

TABLE 2: Amino Acid Substitutions

Original Residue	Exemplary Conservative Substitutions, others are known in the art.
Ala	Ser
Arg	Lys; Gln
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn, Lys
Glu	Asp
Gly	Pro
His	Asn;Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

121. Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g.,

phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

122. For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

123. Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

124. Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

125. It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO:5 sets forth a particular sequence of IL-1ra and SEQ ID NO:9 sets forth a particular sequence of a IL-1R2 protein. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

126. Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

127. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

128. It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

129. As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein in the particular organism from which that protein arises is also known and herein disclosed and described.

130. It is understood that there are numerous amino acid and peptide analogs which can be incorporated into the disclosed compositions. For example, there are numerous D amino acids or amino acids which have a different functional substituent then the amino acids shown in Table 1 and Table 2. The opposite stereo isomers of naturally occurring peptides are disclosed, as well as the stereo isomers of peptide analogs. These amino acids can readily be incorporated into polypeptide chains by charging tRNA molecules with the amino acid of choice and

engineering genetic constructs that utilize, for example, amber codons, to insert the analog amino acid into a peptide chain in a site specific way (Thorson et al., Methods in Molec. Biol. 77:43-73 (1991), Zoller, Current Opinion in Biotechnology, 3:348-354 (1992); Ibba, Biotechnology & Genetic Enginerring Reviews 13:197-216 (1995), Cahill et al., TIBS, 14(10):400-403 (1989); Benner, TIB Tech, 12:158-163 (1994); Ibba and Hennecke, Bio/technology, 12:678-682 (1994) all of which are herein incorporated by reference at least for material related to amino acid analogs).

131. Molecules can be produced that resemble peptides, but which are not connected via a natural peptide linkage. For example, linkages for amino acids or amino acid analogs can include CH_2NH--, --CH_2S--, --CH_2--CH_2 --, --CH=CH-- (cis and trans), --$COCH_2$ --, --$CH(OH)CH_2$--, and --CHH_2SO—(These and others can be found in Spatola, A. F. in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., Vega Data (March 1983), Vol. 1, Issue 3, Peptide Backbone Modifications (general review); Morley, Trends Pharm Sci (1980) pp. 463-468; Hudson, D. et al., Int J Pept Prot Res 14:177-185 (1979) (--CH_2NH--, CH_2CH_2--); Spatola et al. Life Sci 38:1243-1249 (1986) (--CH H_2--S); Hann J. Chem. Soc Perkin Trans. I 307-314 (1982) (--CH--CH--, cis and trans); Almquist et al. J. Med. Chem. 23:1392-1398 (1980) (--$COCH_2$--); Jennings-White et al. Tetrahedron Lett 23:2533 (1982) (--$COCH_2$--); Szelke et al. European Appln, EP 45665 CA (1982): 97:39405 (1982) (--$CH(OH)CH_2$--); Holladay et al. Tetrahedron. Lett 24:4401-4404 (1983) (--$C(OH)CH_2$--); and Hruby Life Sci 31:189-199 (1982) (--CH_2--S--); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is --CH_2NH--. It is understood that peptide analogs can have more than one atom between the bond atoms, such as b-alanine, g-aminobutyric acid, and the like.

132. Amino acid analogs and analogs and peptide analogs often have enhanced or desirable properties, such as, more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others.

133. D-amino acids can be used to generate more stable peptides, because D amino acids are not recognized by peptidases and such. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used to generate more stable peptides. Cysteine residues can be used to cyclize or attach two or more peptides together. This can be beneficial to constrain peptides into particular

conformations. (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992), incorporated herein by reference).

8. Pharmaceutical carriers/Delivery of pharamceutical products

134. The compositions disclosed herein can also be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

135. The compositions can be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like, including topical intranasal administration or administration by inhalant. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

136. Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

137. The materials can be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These can be targeted to a particular cell type via

antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

a) Pharmaceutically Acceptable Carriers

138. The compositions, including antibodies, can be used therapeutically in combination with a pharmaceutically acceptable carrier.

139. Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid

hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

140. Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

141. Pharmaceutical compositions can include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions can also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

142. The pharmaceutical composition can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration can be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed antibodies can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

143. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

144. Formulations for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like could be necessary or desirable.

145. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders could be desirable..

146. Some of the compositions can be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

b) Therapeutic Uses

147. Effective dosages and schedules for administering the compositions can be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms of disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, guidance in selecting appropriate doses for antibodies can be found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York (1977) pp. 365-389. A typical daily dosage of the antibody used alone might range from about 1 μg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above.

148. Following administration of a disclosed composition, such as a vector, for treating, inhibiting, or preventing inflammation, the efficacy of the therapeutic vector can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that a composition, such as a vector, disclosed herein is

efficacious in treating or inhibiting inflammation in a subject by observing that the composition reduces inflammation.

9. Animals

149. Provided herein are transgenic animals comprising germline transmission of any of the vectors or nucleic acids provided herein. In one aspect, the transgenic animal provided herein is an excision activated transgenic (XAT) animal. The disclosed transgenic animals can have temporally and spatially regulated transgene expression (Brooks, AI, et al. 1991. Nature Biotech 15:57-62; Brooks, AI, et al. 1999. Neuroreport 10:337-344; Brooks, AI., et al. 2000. Proc Natl Acad Sci USA 97:13378-13383) of an inflammation element. It is understood that where the transgenic animal comprises a nucleic acid comprising a recombination site, as disclosed herein, delivery of a recombinase, such as Cre recombinase to cells within the provided transgenic animal will result in the expression of the inflammatory modulator, e.g., IL-1β, IL-1ra, COX-2, within those cells.

150. By a "transgene" is meant a nucleic acid sequence that is inserted by artifice into a cell and becomes a part of the genome of that cell and its progeny. Such a transgene an be (but is not necessarily) partly or entirely heterologous (e.g., derived from a different species) to the cell. The term "transgene" broadly refers to any nucleic acid that is introduced into an animal's genome, including but not limited to genes or DNA having sequences which are perhaps not normally present in the genome, genes which are present, but not normally transcribed and translated ("expressed") in a given genome, or any other gene or DNA which one desires to introduce into the genome. This can include genes which are normally be present in the nontransgenic genome but which one desires to have altered in expression, or which one desires to introduce in an altered or variant form. A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that can be necessary for optimal expression of a selected nucleic acid. A transgene can be as few as a couple of nucleotides long, but is preferably at least about 50, 100, 150, 200, 250, 300, 350, 400, or 500 nucleotides long or even longer and can be, e.g., an entire genome. A transgene can be coding or non-coding sequences, or a combination thereof. A transgene usually comprises a regulatory element that is capable of driving the expression of one or more transgenes under appropriate conditions. By "transgenic animal" is meant an animal comprising a transgene as described above. Transgenic animals are made by techniques that are well known in the art. The disclosed nucleic acids, in whole or in part, in any combination, can be transgenes as disclosed herein.

151. Disclosed are animals produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules disclosed herein. Disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the animal is a mammal. Also disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the mammal is mouse, rat, rabbit, cow, sheep, pig, or primate.

152. The disclosed transgenic animals can be any non-human animal, preferably a non-human mammal (e.g. mouse, rat, rabbit, squirrel, hamster, rabbits, guinea pigs, pigs, micro-pigs, prairie dogs, baboons, squirrel monkeys and chimpanzees, etc), bird or an amphibian, in which one or more cells contain heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly, by introduction into a precursor of the cell, such as by microinjection or by infection with a recombinant virus. The disclosed transgenic animals can also include the progeny of animals which had been directly manipulated or which were the original animal to receive one or more of the disclosed nucleic acids. This molecule can be integrated within a chromosome, or it can be extrachromosomally replicating DNA. For techniques related to the production of transgenic animals, see, inter alia, Hogan et al (1986) Manipulating the Mouse Embryo--A Laboratory Manual Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1986).

153. Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Charles River (Wilmington, Mass.), Taconic (Germantown, N.Y.), and Harlan Sprague Dawley (Indianapolis, Ind.). For example, if the transgenic animal is a mouse, many mouse strains are suitable, but C57BL/6 female mice can be used for embryo retrieval and transfer. C57BL/6 males can be used for mating and vasectomized C57BL/6 studs can be used to stimulate pseudopregnancy. Vasectomized mice and rats can be obtained from the supplier. Transgenic animals can be made by any known procedure, including microinjection methods, and embryonic stem cells methods. The procedures for manipulation of the rodent embryo and for microinjection of DNA are described in detail in Hogan et al., Manipulating the Mouse Embryo (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1986), the teachings of which are generally known and are incorporated herein.

154. Transgenic animals can be identified by analyzing their DNA. For this purpose, for example, when the transgenic animal is an animal with a tail, such as rodent, tail samples (1 to 2 cm) can be removed from three week old animals. DNA from these or other samples can

then be prepared and analyzed, for example, by Southern blot, PCR, or slot blot to detect transgenic founder (F (0)) animals and their progeny (F (1)and F (2)). The present invention further provides transgenic non-human animals that are progeny of crosses between a transgenic animal of the invention and a second animal. Transgenic animals can be bred with other transgenic animals, where the two transgenic animals were generated using different transgenes, to test the effect of one gene product on another gene product or to test the combined effects of two gene products.

155. The provided compositions can be evaluated using a mouse model of arthritis. As prolonged expression of IL-1β in the joint can lead to the development of arthrosis similar to that seen in arthritis patients, disclosed is a mouse model of arthritis based on prolonged, low level intra-articular transgenic expression of IL-1β. The role of IL-1β, TNFα and other inflammatory mediators, such as prostanoids, are well recognized in the pathogenesis of arthritis. The two most commonly forms of arthritis are osteoarthritis (OA), which affects about 80%-90% of all adults over the age of 65, and rheumatoid arthritis (RA), which affects approximately 1% of the general U.S. population. Although distinct differences exist between OA and RA, both appear to develop secondary to a pro-inflammatory cascade. Previous animal models have proven valuable in studying arthritis and testing novel therapies, including the model of methylated bovine serum albumin/IL-1β, intra-articular administration of IL-1β, constitutive intra-articular expression of IL-1β following ex vivo transfer of genetically engineered synoviocytes, as well as the TNFα transgenic mouse model. The aforementioned IL-1β models are based on the direct administration of a deleterious agent, whereas the TNFα transgenic mouse is based on the prolonged expression of TNFα in vivo and has thus far yielded valuable insight on the role of TNFα in the development of arthritis. However, as with the majority of transgenic mice, TNFα transgenesis is susceptible to uncontrolled and uncharacterized developmental compensatory changes.

156. The provided mouse model is based on a method (somatic mosaic analysis) utilizing a germline transmitted recombinational substrate containing a dormant transcription unit and somatic gene transfer of a viral vector that expresses Cre recombinase that "activates" the gene of interest. IL-1β excisionally activated transgenic (IL-1β^{XAT}) mice, and variations thereof, have been generated using this method. The provided mouse model is the subject of U.S. Patent Application No. 60/627,604, which is herein incorporated by reference in its entirety. This mouse model allows for the induction of localized inflammation based on the delivery of a Cre recombinase expression vector such as FIV(Cre) to the target site. Variations include the use of

cell or tissue specific promoters such as in for example the COL1A1-IL-1β^{XAT} mouse. For example, the delivery of FIV(Cre) to, for example, the joints of the COLL1A1-IL-1β^{XAT} mouse can induce inflammation to model arthritis. This mouse model can thus be used to, for example, test or optimize the effects of the provided constructs on arthritis. As another example, delivery of FIV(Cre) to the circulation or joint of the COLL1A1-IL-1β^{XAT} mouse can induce inflammation in the brain to model, for example, Alzheimer's disease.

157. IL1β^{XAT} regulation is controlled in a temporal (time) and spatial (location) fashion by the Cre/loxP molecular genetic method utilizing (1) a germline transmitted recombinational substrate (e.g. COLL1-IL1β^{XAT}) containing a dormant transcription unit and (2) somatic gene transfer of a viral vector that expresses Cre recombinase which "activates" the gene of interest. Thus, these mice can be used herein to induce IL-1β constitutive expression in the joints (e.g., knee) of mice. As an example, localized transgene activation, i.e., IL-1β, can be accomplished in IL-1β^{XAT} mice by the intracapsular injection of FIV(Cre), a lentivirus capable of transducing soft and hard tissues of joints, to the area of interest, and subsequent recombinational excision of the ►STOP► cassette leading to gene transcription. Recombination-mediated gene "activation" permanently alters the genetic constitution of infected cells thus allowing chronic IL-1β synthesis. The COLL1A1 promoter can further be used to target gene expression to chondrocytes, osteocytes and fibroblasts, making this transgenic mouse available for the study of arthritis in any joint of interest. This promoter has been shown to target gene expression in bone and cartilage and was cloned in the IL-1β^{XAT} gene in place of the CMV promoter (Figure 1): (COLL1A1-IL1β^{XAT}) COLL1A1 => ►STOP► ssIL1β – IRES – lacZ

158. COLL2 is another suitable promoter. This transgene has been constructed and tested in a murine NIH 3T3 stable cell line following expression of Cre recombinase by the transient transfection of the pRc/CMV-CreWT expression vector or after infection by the lentiviral vector FIV(Cre).

159. The somatic gene transfer of the recombinase, such as Cre can be performed using any type of vector system producing the recombinase. However, in certain embodiments, the vector system is a self inactivating vector system, wherein the promoter, for example, of the recombinase is flanked by recombination sites so that upon production of the recombinase, the recombinase will down regulate its own production. The delivery vectors for the recombinase can be CRE mediated.

160. For example, activation of the dormant COLL1- IL1β^{XAT} can be mediated by the transfer of Cre recombinase to the area of interest (e.g. knee) via a self-inactivating Cre feline immunodeficiency virus FIV(Cre). The effects of this FIV vector system have been previously examined using the reporter gene lacZ (β-galactosidase) in mice that received intra-articular injections of a viral solution [Kyrkanides S, *et al.* (2004). *J Dental Res* 83: 65-70], wherein transduction of soft (articular disc) and hard (cartilage) TMJ tissues was demonstrated. The FIV(Cre)vector has been constructed by cloning a loxP-flanked ("floxed") nlsCre cassette in the place of the lacZ gene; the nuclear localization signal (nls) was fused to the *cre* open reading frame by PCR and subsequently cloned into the TOPO 2.1 vector (Invitrogen) per manufacturer's instructions employing a custom-made floxed cloning cassette. The reason for developing a self-inactivating *cre* gene is based on a recent paper [Pfeifer A and Brandon EP, Kootstra Neeltje, Gage FH, Verma IM (2001). Proc Natl Acad Sci U.S.A. 98: 11450-5], whereby the authors reported cytotoxicity due to prolonged expression of Cre recombinase mediated by infection using a lentiviral vector. In the provided construct, upon production of adequate levels of Cre recombinase to produce excisional activation of COLL1-IL1β^{XAT} following successful transduction of target cells with FIV(Cre), Cre is anticipated to de-activate the *cre* gene by loxP-directed self excisional recombination.

10. Kits

161. Disclosed herein are kits that are drawn to reagents that can be used in practicing the methods disclosed herein. The kits can include any reagent or combination of reagent discussed herein or that would be understood to be required or beneficial in the practice of the disclosed methods. For example, the kits could include primers to perform the amplification reactions discussed in certain embodiments of the methods, as well as the buffers and enzymes required to use the primers as intended.

D. Methods of making the compositions

162. The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

1. Nucleic acid synthesis

163. For example, the nucleic acids, such as, the oligonucleotides to be used as primers can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for example, Sambrook *et al.*,

Molecular Cloning: A Laboratory Manual, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Biosearch, Burlington, MA or ABI Model 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta *et al.*, *Ann. Rev. Biochem.* 53:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang *et al.*, *Methods Enzymol.*, 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen *et al.*, *Bioconjug. Chem.* 5:3-7 (1994).

2. Peptide synthesis

164. One method of producing the disclosed proteins, such as SEQ ID NO:5, is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (*tert* -butyloxycarbonoyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to the disclosed proteins, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form an antibody, or fragment thereof. (Grant GA (1992) Synthetic Peptides: A User Guide. W.H. Freeman and Co., N.Y. (1992); Bodansky M and Trost B., Ed. (1993) Principles of Peptide Synthesis. Springer-Verlag Inc., NY (which is herein incorporated by reference at least for material related to peptide synthesis). Alternatively, the peptide or polypeptide is independently synthesized *in vivo* as described herein. Once isolated, these independent peptides or polypeptides can be linked to form a peptide or fragment thereof via similar peptide condensation reactions.

165. For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen L et al., Biochemistry, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method consists of a two step

chemical reaction (Dawson et al. Synthesis of Proteins by Native Chemical Ligation. Science, 266:776-779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide--thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site (Baggiolini M et al. (1992) FEBS Lett. 307:97-101; Clark-Lewis I et al., J.Biol.Chem., 269:16075 (1994); Clark-Lewis I et al., Biochemistry, 30:3128 (1991); Rajarathnam K et al., Biochemistry 33:6623-30 (1994)).

166. Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al. Science, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et al., Techniques in Protein Chemistry IV. Academic Press, New York, pp. 257-267 (1992)).

3. Processes for making the compositions

167. Disclosed are processes for making the compositions as well as making the intermediates leading to the compositions. There are a variety of methods that can be used for making these compositions, such as synthetic chemical methods and standard molecular biology methods. It is understood that the methods of making these and the other disclosed compositions are specifically disclosed.

168. Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a promoter element and a nucleic acid element disclosed herein. The nucleic acid element can, for example, encode a ligand binding inhibitor. Thus, disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a promoter element and an IL-1ra element. Also disclosed is a nucleic acid molecule produced by the process comprising linking in an operative way a promoter element and an IL-1R2 element. Also disclosed is a nucleic acid molecules produced by the process comprising linking in an operative way a promoter element and an IL-1R1 fragment element. Also disclosed is a nucleic acid molecules produced by the process comprising linking in an operative way a promoter element and an IL-1 fragment element.

169. Also disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a promoter element and a nucleic acid element wherein the nucleic acid encodes a gene expression inhinitor disclosed herein. As an example, disclosed are nucleic

acid molecules produced by the process comprising linking in an operative way a promoter element and a COX-1 siRNA element. Also disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a promoter element and a COX-2 siRNA element. Also disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a promoter element and a mPGES siRNA element. Also disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a promoter element and cPGES siRNA element.

170. Further disclosed are cells produced by the process of transforming the cell with any of the disclosed nucleic acids. Disclosed are cells produced by the process of transforming the cell with any of the non-naturally occurring disclosed nucleic acids.

171. Disclosed are any of the peptides produced by the process of expressing any of the disclosed nucleic acids. Disclosed are any of the non-naturally occurring disclosed peptides produced by the process of expressing any of the disclosed nucleic acids. Disclosed are any of the disclosed peptides produced by the process of expressing any of the non-naturally disclosed nucleic acids.

172. Disclosed are animals produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules disclosed herein. Disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the animal is a mammal. Also disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the mammal is mouse, rat, rabbit, cow, sheep, pig, or primate. Also disclosed are mammals wherein mammal is a murine, ungulate, or non-human primate.

173. Also disclose are animals produced by the process of adding to the animal any of the cells disclosed herein.

E. Methods of using the compositions

1. Methods of using the compositions as research tools

174. The disclosed compositions can be used in a variety of ways as research tools. For example, the disclosed compositions, such as SEQ ID NOs:5 can be used to study the interactions between IL-1 and IL-1R1, by for example acting as inhibitors of binding.

2. Therapeutic Uses

175. Effective dosages and schedules for administering the compositions can be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the

desired effect in which the symptoms of the disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products.

176. Following administration of a disclosed composition, such as the disclosed constructs, for treating, inhibiting, or preventing inflammation, the efficacy of the therapeutic construct can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that a composition, such as the disclosed constructs, disclosed herein is efficacious in treating inflammation or inhibiting or reducing the effects of inflammation in a subject by observing that the composition reduces the onset of the conditions associated with these diseases. Furthermore, the amount of protein or transcript produced from the constructs can be analyzed using any diagnostic method. For example, it can be measured using polymerase chain reaction assays to detect the presence of construct nucleic acid or antibody assays to detect the presence of protein produced from the construct in a sample (e.g., but not limited to, blood or other cells, such as neural cells) from a subject or patient.

F. Examples

177. The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the disclosure. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

1. Example 1 - Construction of an inducible Interleukin-1β transgene - IL-$1\beta^{XAT}$

178. The human IL-1β cDNA was cloned from human U937 cells (ATCC, Manassas VA) by polymerase chain reaction (PCR) as follows: Total mRNA was extracted employing the TRIzol® reagent (Invitrogen, Carlsbad CA) per manufacturer's instructions. PCR primers were designed for the amplification of the portion of the cDNA that corresponds to the mature,

secreted IL-1β protein. The peptide secretion signal (ss) of the human IL-1 receptor antagonist gene (IL1-RA) was incorporated into the upper PCR primer, upstream to the IL-1β open reading frame (ORF), to ensure proper compartmentalization and secretion of the transgenic IL-1β peptide. This PCR-synthesized ss-IL1β fusion construct was cloned directly into the TOPO 2.1 vector (Invitrogen) per manufacturer's instructions. Subsequently, the cell autonomous and gratuitous β-galactosidase reporter gene (lacZ) was inserted down-stream to the IL-1β ORF, followed by the bovine growth hormone poly A tail (pA) sequence: ssIL-1β-IRES-lacZ bicistronic gene (Fig. 1). Translation of the second ORF, lacZ, is facilitated by an internal ribosomal entry sequence (IRES). During the initial stages of experimentation, expression of the bicistronic ssIL-1β-IRES-lacZ transgene was ubiquitously driven by the cytomegalovirus promoter (CMV), the transcription of which was inhibited by a loxP-flanked (floxed) transcriptional termination cassette STOPfl/fl. Transcriptional activation and transgene expression can be turned-on by loxP-directed DNA recombination mediate by the bacteriophage P1 Cre recombinase (Cre/loxP system).

a) Cre-mediated activation of the inducible IL-1β^{XAT} transgene

179. The function of IL-1β^{XAT} was tested in vitro by two different experimental strategies. First, IL-1β^{XAT} regulation by Cre recombinase was evaluated in NIH 3T3 murine fibroblasts (ATCC) in vitro. The IL-1β^{XAT} gene was transiently co-expressed with the wild type cre gene (cloned into the expression vector pRc/CMV-CreWT; Invitrogen) following transient transfection using the LIPOFECTAMINE 2000 reagent (Invitrogen) per manufacturer's instructions. As anticipated, transient expression of Cre recombinase resulted in loxP-directed DNA recombination of IL-1β^{XAT} and excision of the "floxed" transcriptional termination signal ►STOP►, ultimately leading to gene activation. Control conditions included co-transfection of IL-1β^{XAT} with the expression vector pRc/CMV- (lacking any gene), as well as naïve NIH 3T3 cells. IL-1β expression was assessed at the mRNA level by reverse transcriptase PCR (RT-PCR), and lacZ expression was evaluated by X-gal histochemistry. In brief, no IL-1β transcript was detected in naïve NIH 3T3 cells; in contrast, IL-1β^{XAT} + Cre co-transfection resulted in induction of ssIL-1β and lacZ gene expression (Fig. 2). In addition, IL-1β^{XAT} function was evaluated in the inducible Cre recombinase cell line, 293HGLVP/CrePr, a stable cell line recently developed for testing the regulation of excisionally-activated genes utilizing the Cre/loxP technology and described in U.S. Patent Application No. 10/978927, herein incorporated by reference). This cell line comprises an inducible, loxP-directed DNA recombination system by placing Cre recombinase under dual transcriptional and post-translational control (Kyrkanides et al., 2003,

herein incorporated by reference). In brief, the system is comprised of two components: (1) The chimeric transcriptional activator GLVP and (2) the CrePr fusion protein, which consists of the bacterial Cre recombinase and the mutated progesterone receptor hPR891 gene, driven by a custom GAL45/TATA minimal promoter. The mutated hPR891 receptor is highly sensitive to the synthetic progesterone compound mifepristone (RU486). Binding of RU486 to hPR891 results in activation of GLVP and subsequent synthesis of CrePr, the activity of which is also turned-on by RU486 at the post-translational level. RU486 administration to 293HGLVP/CrePr cells following IL-1β^{XAT} transfection resulted in DNA excisional recombination and subsequent expression of human IL-1β and the bacterial β-galactosidase reporter gene (lacZ). Please refer to Figure 3 for summary of the experiment.

b) IL-1β^{XAT} activation produces a biologically potent IL-1β cytokine

180. The ability of murine cells to synthesize and secrete biologically active IL-1β cytokine was tested in vitro as follows. In an experiment similar to that described in Figure 2, murine NIH 3T3 fibroblasts were transfected with the IL-1β^{XAT} gene. Concomitantly, Cre recombinase was transiently expressed in these cells (co-transfection of the pRc/CMV-CreWT vector), and the conditioned supernatant media were collected at 72 hours. The presence of human IL-1β was confirmed by ELISA. The conditioned media were then placed on naïve murine fibroblasts, and levels of the inducible cyclooxygenase COX-2 were evaluated as a measure of cytokine potency by quantitative RT-PCR in total mRNA extracts using protocols previously described [Moore, AH, et al. Journal of Neuroimmunology. 148(1-2):32-40, 2004]. Control experimental conditions included conditioned media derived from cells co-transfected with the pRc/CMV- backbone vector (lacking the cre gene) along with the IL-1β^{XAT} gene, as well as naïve cells. In brief, murine fibroblasts treated with conditioned medium collected from Cre-activated IL-1β^{XAT} cells resulted in significant COX-2 induction compared to cells exposed to media derived from pRc/CMV-treated or naïve cells. Please see Figure 4 for a summary of the experiment.

c) IL-1β induces down-stream inflammation-related genes

181. IL-1β is a multi-potent pro-inflammatory cytokine, the expression of which is rapidly upregulated following trauma and/or inflammation. Moreover, a plethora of inflammation-related genes are in turn induced by IL-1β, leading to exacerbation of the inflammatory response. IL-1β regulates down-stream inflammatory genes, including the inducible isoform of cyclooxygenase (COX-2) and the intercellular adhesion molecule-1 (ICAM-1), the monocyte chemoattractant protein-1 (MCP-1), as well as collagenases A (MMP-2) and B

(MMP-9). ICAM-1 and MCP-1 are molecules associated with the recruitment of circulating immune cells at the site of injury (i.e. neutrophils and monocytes, respectively), whereas MMP-2 and MMP-9 are collagenases associated with tissue destruction during arthritis and injury. Primary rat endothelial cells were employed as a representative rodent model to investigate the effects of IL-1β on the regulation of ICAM-1, MCP-1, MMP-2 and MMP-9 at the transcriptional level (mRNA) as well as the enzyme activity level (zymography). Figure 5 summarizes the regulation of these genes over time. IL-1β upregulated the synthesis of COX-2, MCP-1, ICAM-1 and the inducible collagenase B (MMP-9). mRNA levels of the constitutive collagenase B (MMP-2) were not altered by IL-1β, but interestingly MMP-2 enzyme activity also increased with time, presumably due to post-translational activation from other MMPs.

d) The COLL1A1 promoter drives IL-1β^{XAT} expression to collagen I producing cells

182. Prolonged expression of IL-1β in the joint can lead to the development of arthrosis similar to that seen in arthritis patients. This can be demonstrated using temporally and spatially controlled expression of IL-1β in mice, which can be accomplished by targeting IL-1β^{XAT} transgene expression to chondrocytes, osteocytes and fibroblasts by the 3.6 Kb promoter of the A1 chain of pro-collagen 1 gene. This promoter has been shown to target gene expression in bone and cartilage and was cloned in the IL-1β^{XAT} gene in place of the CMV promoter (Figure 1): (COLL1A1-IL1β^{XAT}) COLL1A1 => ▶STOP▶ssIL1β – IRES – lacZ

183. The collagen 1 promoter was chosen based on the prediction that FIV(Cre) would primarily infect cells located superficially within the joint capsule, including the meniscus, cartilage, and perhaps bone (Kyrkanides et al., 2004). COLL2 is another suitable promoter. This transgene has been constructed and tested in a murine NIH 3T3 stable cell line following expression of Cre recombinase by the transient transfection of the pRc/CMV-CreWT expression vector or after infection by the lentiviral vector FIV(Cre). Expression of Cre recombinase led to transgene activation and IL-1β expression. Please refer to Figure 6 for summary of the experiment.

e) Transgene activation in joints of Col1-IL1β^{XAT} mice

184. In order to evaluate the effect of transgene activation in the joints of Col1-IL1β^{XAT} mice, two sets of Col1A1-IL1β^{XAT} mice received intra-articular FIV(Cre) injections (a total of 10^6 infectious particles) in the right and left knee, as well as the left and right temporomandibular joint (TMJ). The mice were monitored over a period of 8 weeks for changes

in grooming behavior and locomotion. The mice were subsequently sacrificed and their knees and TMJs were histologically analyzed.

185. Behavioral changes were assessed as previously described (Dubuisson, D. and Dennis, S. Pain 1977; 4: 161-74; Abbott FV, et al. Eur J Pharmacol 1986; 126:126–41), which are herein incorporated by reference for teachings related to these methods. In brief, a group of Col1-IL1β^{XAT} transgenic mice (N=3) received a single intra-articular injection of 10^6 infectious particles of FIV(Cre) in the right and left knees at 2 months of age. In addition, a second group of mice (N=3) received saline injection and served as controls. During a session, each mouse was videotaped for 1 hour. The tape was then transferred digitally to a computer and analyzed in 20 periods of 3 minutes each. The duration of each mouse displaying grooming and licking was recorded and summed as seconds by an investigator who was blind to the animal group assignment. Injection of FIV(Cre) into the knee of Col1-IL1β^{XAT} transgenic mice resulted in a four-fold increase in the duration of grooming as compared to saline-injected controls (Fig. 9, $P<0.05$).

186. Four groups of mice (N=3) were evaluated in terms of locomotive behavior by the rotorod appliance (Columbus Instruments, Columbus OH) and the lapse time until the mice fell off the rotating cylinder (20 rpm) was recorded. The mice were evaluated over a period of 8 weeks following the intra-articular injections (8 wks – 16 wks of age). As seen in Figure 10, it was demonstrated that FIV(Cre)-injected Col1-IL1β^{XAT} transgenic mice developed significant locomotive deterioration (Tg+Cre) compared to transgenic mice injected with the control FIV(gfp) vector (TG+gfp), as well as the other control animals groups (WT-Cre & WT-saline).

187. Immunocytochemical detection of the reporter gene β-galactosidase was employed to confirm the activation of the Col1-IL1β^{XAT} transgene by FIV(Cre) in this mouse model using antibodies raised against β-galactosidase and Cre recombinase. Shown in Figure 11 is FITC-conjugated immunodetection of β-galactosidase (Fig. 11A), Texas Red-conjugated immunodetection of Cre recombinase (Fig. 11B), B/W image of the same microscopic field (Fig. 11C), overlap of panels A+B (Fig. 11D), and overlap of panels A+B+C (Fig. 11E). Demonstrated is the co-expression of β-galactosidase and Cre recombinase *in vivo* (Fig. 11, solid arrows). Note that there are more red cells than green cells (Fig. 11, open arrows) indicating that not all infected cells express the transgene Col1A1→IL1β-IRES-lacZ in the same capacity.

188. H&E staining of a knee section harvested from a 4 month old Col1-IL1β^{XAT} transgenic mouse injected with FIV(Cre) revealed the formation of fibrillations (Fig. 12A, solid arrow) and of an articular lip (Fig.12B, open arrow). In contrast, a transgenic mouse that

received the control vector FIV(GFP) did not develop such anatomic aberrations (Fig. 12B). Alcian blue / orange semi-quantitative evaluation showed a decrease in cartilage (Fig. 12C, less blue stain) and bone (Fig. 12D, less red stain) density in the Col1-IL1β^{XAT}+FIV(Cre) knees compared to controls (Fig. 12E). Moreover, increased cloning along with thickening of the articular surfaces was observed in the experimental animals (Fig.12C, indicated by small arrows). These observations indicate the presence of arthritis in the knee following transgene induction by Cre recombinase.

189. Eight weeks after FIV(Cre) injection in the knee and TMJ of Col1-IL1β^{XAT} mice, the brain was evaluated for activation of microglia and astrocytes by immunocytochemistry. Using a monoclonal antibody raised against the MHC-class II antigen, the presence of activated microglia was detected in the brain (Fig. 13A,C). In contrast, control animals did not display any MHC-II positive cells. Interestingly, there was lack of astrocyte activation in the brains of these animals as assessed by glial fibrillary acidic protein (GFAP) (Fig. 13B,D). In general, control animals (inactive transgenic mice) displayed no signs of brain inflammation by MHC-II or GFAP immunocytochemistry.

190. Eight weeks after FIV(Cre) injection in the TMJ of Col1-IL1β^{XAT} mice anatomic aberrations of the joint were evaluated by semi-quantitative Alcian blue – orange G histochemistry. Shown in Figures 14A and C are a TMJ section from an inactive Col1-IL1β^{XAT} mouse depicting the condylar head as well as the meniscus. In comparison, Figures 14B and C depict a TMJ section harvested from a Col1-IL1β^{XAT} mouse injected with FIV(Cre) in the TMJ. An apparent reorganization of the TMJ cell layers was observed following FIV(Cre) injection, whereby a loss of the most superficial cell layer was noted accompanied by disorganization of the proliferative layer of chondrocytes (Fig. 14, open arrows). In addition, a decrease in cartilage content was observed in the condylar head of FIV(Cre)-treated Col1-IL1β^{XAT} mice as evaluated semi-quantitatively by Alcian blue – orange G histochemistry (Fig. 14).

2. Example 2 - Development of arthritis in the adult mouse: the IL-1β somatic mosaic model

a) Development of the IL-1B somatic mosaic mouse

191. Prolonged, low-level intra-articular expression of IL-1β in the adult mouse can result in the development of arthritis. This can be demonstrated using the somatic mosaic analysis method for the induction of long-term expression of IL-1β, due to a permanent change in the genetic constitution of infected cells, at a particular location and during a specific developmental stage. The somatic mosaic analysis model offers significant advantages compared

to traditional transgenic mice, because it avoids compensatory adaptations often encountered in transgenic mice during development and allows regional activation of a gene (Brooks et al., 1997, 1999). The collagen I promoter was chosen based to drive transgene expression based on the prediction that FIV(Cre) would primarily infect cells located superficially within the joint capsule, including the meniscus, cartilage, and perhaps cancellous osteocytes. COLL2 is another choice of suitable promoter.

192. COLL1-IL1β^{XAT} was microinjected in fertilized C57BL/6 oocytes and subsequently implanted into pseudo-pregnant mothers. One set of micro-injection has already been performed that yielded 8 pups, of which 3 were identified as positive founders by PCR of genomic DNA extracted from tail snips employing primers specifically designed against the IL1β^{XAT} transgene. The 3 founders have been bred with C57Bl/6 wild type stock mice for analysis of germ-line transmission of a functional transgene (Ngo et al., 2002; Pinkert 2003). In fact, at least two founders have successfully given transgenic pups at the time of grant submission, which were being raised to maturity for further breeding.

b) Characterize the activation of COLL1-IL1β^{XAT} in vivo

193. COLL1-IL1β^{XAT} function can be evaluated in transgenic lines after FIV(Cre) injection into the knee joint capsule (approximately 10^7 infectious particles in 50µl volume) in 3 month old mice and activation of the dormant COLL1- IL1β^{XAT}. The ability of FIV(Cre) to activate the dormant COLL1-IL1β^{XAT} gene has been previously described in U.S. Patent Application No 60/627,604, herein incorporated by reference for this teaching. COLL1-IL1β^{XAT} activation can be evaluated as follows. First, lacZ expression can be readily assessed in decalcified histology sections by X-gal histochemistry and immunocytochemistry as previously described (Kyrkanides et al., 2001, 2004). ssIL-1β and lacZ transcript levels can be assessed by semi-quantitative RT-PCR in total mRNA knee extracts from experimental FIV(Cre) intra-articular injection and control FIV(lacZ) intra-articular injection mice. Localization of ssIL-1β and β-galactosidase can be achieved on histology sections by immunocytochemistry using antibodies raised specifically against bacterial β-galactosidase and human IL-1β; the identity of transduced cells can be confirmed by double immunofluorescence employing antibodies raised against the following antigens. Osteocytes/osteblasts can be confirmed by the expression alkaline phosphatase, osteocalcin, type I collagen. Chondrocytes can be confirmed by detection of collagen II (Scott-Burden et al., 2002). Histologically, ssIL-1β mRNA localization can be performed by in situ hybridization (ISH); the identity of transduced cells can be confirmed by coupling ISH with immunocytochemistry (ICC), employing aforementioned antibodies. The

levels of secreted human IL-1β in joint synovial fluid can be analyzed by ELISA as described in Example 1 (Catalog # DLB50; R&D Systems Inc, Minneapolis MN). 3-5 mouse lines can be analyzed for transgene function at the mRNA, protein and histology levels following gene activation. For this purpose, transgenic mice can be injected intra-articularly with FIV(Cre), FIV(lacZ) or saline at 2 months of age and subsequently analyzed 4 weeks later. Two mouse lines would result, one expressing "high" and another with "moderate" levels of IL-1β that can be further analyzed.

c) Investigate the long-term effects of IL-1B expression in the knee

194. The effects of IL-1β expression in the knee can be studied in young adult (3 month old) COLL1-IL1β^{XAT} transgenic mice over time (4-8-12-16 weeks) after intra-articular injection of FIV(Cre), FIV(lacZ) or saline. Intra-articular transfer of Cre recombinase to the knee of COLL1-IL1β^{XAT} transgenic mice can result in sustained expression of human IL-1β by infected cells. In contrast, FIV(lacZ)-injected mice would lack detectable human IL1β expression. Saline-treated mice can also serve as controls. After intra-articular injections, the animals can be returned to their cages. Subsequently, the effects of FIV(Cre), FIV(lacZ) and saline intra-articular injection can be analyzed in the two COLL1-IL1β^{XAT} transgenic mouse lines identified above: one characterized by the highest expression of IL-1β among the mouse lines analyzed (as determined by ELIZA in knee homogenates) following FIV(Cre) intra-articular injection ("high"), and a second mouse line with median expression of IL-1β ("moderate"). The effects of IL-1β expression can be evaluated over time (4-8-12-16 weeks).

d) Clinical - Behavioral evaluation

195. Arthralgia and joint dysfunction (knee pain) can be measured by (1) Locomotive performance, as evaluated on a rotating cylinder (rotorod appliance, Columbus Instruments, Columbus OH) and (2) Muscle strength, as evaluated by the inverted mesh method. In brief, this method evaluates neuromuscular condition by assessing grip strength. A clear plastic cylinder (20 cm x 20cm x 30 cm) that was covered on the one end by a wire mesh has been constructed. The mesh wire bars were 1 mm in diameter and 1 cm apart. A rectangular area of the screen was taped so that the animals were confined in the center of the mesh. After the mice were placed on the screen, the cylinder was turned up-side-down over bedding: the lapse time until their fall from the mesh was recorded in seconds. If a mouse fell in less than 10s in the first try, this animal was given a second chance. These methods have been adopted from clinical experience and aim at replicating behavioral and somatic events seen in human patients with arthralgia. The total lapse time until the mouse falls off the mesh can thus be recorded.

e) Peripheral evaluation

196. Since IL-1β is a multipotent cytokine known to induce a number of inflammation-related genes, the expression of cytokines (TNFα, IL-6, murine IL-1β), adhesion molecules (ICAM-1, VCAM-1), chemokines (MCP-1), and collagenases (MMP-3, MMP-9) can be evaluated at the mRNA and protein level. In addition, the levels of the inducible COX-2, COX-1, mPGES and cPGES can be measured at the mRNA and protein levels as previously described (REF), as well as production of prostaglandin PGE_2. Moreover, joint morphology can be assessed in H&E-stained histology sections as follows. Degenerative changes in the articular cartilage can be evaluated and graded in sagittal sections examined under light microscope, and scored into five categories: grade 0, no apparent changes; grade 1, superficial fibrillation of articular cartilage; grade 2, defects limited to uncalcified cartilage; grade 3, defects extending into calcified cartilage; and grade 4, exposure of subchondral bone at the articular surface. Each joint can be graded according to the highest score observed within the serial sections. The presence of inflammatory cells, including neutrophils, monocytes/macrophages and lymphocytes in the joint can be investigated at the histology level by immunocytochemistry and double immuno-fluorescence in experimental and control mice sacrificed 4-8-12-16 weeks after treatment. In brief, neutrophils can be detected by a rat anti-murine neutrophil antibody (MCA771GA; Serotec, Raleigh, NC); monocytes & macrophages can be stained with a rat anti-mouse CD11b antibody (MC A74; Serotec Inc); activated cells can be immunolocalized by a rat anti-major histocompatibility complex class-II antibody (MHC-II; Bachem, Torrance, CA; clone ER-TR3). Lymphocytes can be detected by a monoclonal antibody raised against CD3 (MCA 1477; Serotec) and CD4 (Serotec) Quantification of the number of cells can be described both in terms of number of positive cells per field, as well as staining profile. Endothelial cells can be detected by antibodies raised against PECAM-1 (CD31) (Kyrkanides et al. 2003).

197. Since the introduction of FIV proteins can elicit an immunologic response in mice treated with FIV vectors, the host's immunologic response can be characterized following FIV intra-articular injection. The presence (titers) of antibodies against viral and transgenic proteins can be quantitatively assessed in blood serum at the different experimental time points. To this end, IgG and IgM titers for the FIV p24 antigen as well as human IL-1β can be assessed by customized ELISA method. In brief, ELISA plates can be coated with 5 μg of human IL-1β (Sigma; St. Louis MO) or p24 recombinant proteins (IDEXX Laboratories Inc.; Westbrook ME). After incubation with the test sera, the plates can be incubated with alkaline phosphatase-conjugated goat anti-mouse IgG and IgM (Southern Biotechnology Associates, Inc; Birmingham

ALJ. Antibody titers can be established as the serum dilution that reached absorbance levels (at 405nm) of saline injected mice assuming linear extrapolation (Kang et al., 2002).

f) Central nervous system evaluation

198. A number of small neuropeptides, such as substance P (SP) and calcitonin-gene related peptide (CGRP), have been implicated in the transmission of pain from the periphery to the central nervous system (CNS). Sustained expression of IL-1β in the mouse knee could elicit, in addition to a peripheral inflammatory response, changes in the expression of neurotransmitters in the CNS. Therefore, the expression of SP and CGRP can be evaluated in the spinal cord of experimental and control mice adapting methods previously described (Kyrkanides et al. 2002a, 2002b). Particular emphasis can be given to the region of the spinal cord where nociceptive afferents from the knee synapse at the dorsal horn of the spinal cord.

g) Results and alternatives

199. Intra-articular injection of FIV(Cre) can result in expression of IL-1β: FIV-mediated Cre recombinase expression in the joint can permanently alter the genetic constitution of infected cells in the knee and result in intra-articular expression of IL-1β. Previous work with FIV demonstrated successful infection of soft and hard articular tissues by the virus following intra-articular injection (Kyrkanides S., et al. Journal of Dental Research. 83(1):65-70, 2004, herein incorporated by reference for this teaching). FIV(Cre) injections can also be repeated to COLL1-IL1β^{XAT} transgenic mice, allowing the animals to survive for 26 weeks, at which time they could be sacrificed and analyzed as previously described. The COLL1-IL1β gene can also be cloned into the FIV backbone vector using established molecular biology methods to develop FIV(COLL1-IL1β), a virus capable of transducing cells with a COLL1A1-driven ssIL-IL1β. Moreover, a stronger promoter, such as the chicken β-actin / CMV fusion promoter (Daly et al., 1999) can be employed to drive ssIL-1β in the knee following intra-articular injection of the new FIV(COLL1-IL1β) vector. This is a viable alternative to the somatic mosaic model. To this end, expression of the reporter gene β-galactosidase has been observed for up to 5 weeks in the knee (Kyrkanides et al., 2004), and up to 3 months in the liver and brain in vivo. The advantage of the somatic mosaic model is that FIV(Cre) injection will result in a permanent alteration of the cell genome and lead to a chronic low-level inflammation similar to that observe in human patients.

3. Example 3 – Small inhibitory siRNA-based treatment for the management of arthrtis

200. IL-1β is an inducer of cyclooxygenase-2 (COX-2), a key rate-limiting enzyme in the production of prostanoids during inflammation. COX-2 is of particular therapeutic interest

since it is the target of commercially available over-the-counter and prescription drugs often utilized in cases of arthralgia. Small inhibitory RNA (siRNA) constructs have been developed that are capable of attenuating COX-2, as well as other members of the cyclooxygenase-prostaglandin pathway, including mPGES and cPGES. These siRNA constructs can be expressed from a feline immuno-deficiency viral platform, for example, FIV(siRNA), and can be used for gene therapy for the treatment of arthritis. Therefore, FIV(siRNA) transfer vectors can be generated for COX-2, the constitutive isoform COX-1, as well as cPGES and mPGES. To this end, joint pathology and behavior can be investigated in IL-1β^{XAT} mice treated with FIV(siRNA) at various time points after induction of arthritis, and compared to mice treated with pharmacologic selective inhibitors for COX-2 and COX-1, as well as a mixed inhibitor (i.e. ibuprofen).

a) Anti-inflammatory regimen in COLL1Pr-IL1β^{XAT} transgenic mice

201. It has been established that IL-1β drives the expression of COX-2 to form prostaglandin E_2 (PGE_2), a principal mediator of inflammation in a number of tissues, including joints. COX-2, as well as the constitutively expressed COX-1, an be temporally (time course) and spatially (sites of expression) characterized at the molecular level, and can be correlated with PGE_2 levels and other inflammatory mediators related to arthritis, as well as neurotransmitter expression and behavioral measures in the IL-1β^{XAT} transgenic mice. Transgenic mice of the founder line identified in Example 2 can be treated with FIV(siRNA) for COX-1, COX-2, mPGES and cPGES. In addition, other groups of mice can be given a pharamocologic inhibitor for COX-2 (NS-398) as well as the NSAID ibuprofen in the chow. In brief, knee inflammation can be induced in 8 weeks old COLL1-IL1β^{XAT} transgenic mice by intra-articular injection of FIV(Cre). In keeping with the clinical use of anti-inflammatory regimes, anti-inflammatory treatment can be initiated at a time when the FIV(Cre)-injected mice begin to demonstrate knee joint pathology and dysfunction (based on the data derived from Example 2). Alternatively, anti-inflammatory treatment can begin at a set time before or after the FIV(Cre) injection. The mice can be sacrificed at various time points following initiation of anti-inflammatory treatment (4-8-12-16 weeks). Consequently, the effects of therapy can be characterized on knee arthritis (anatomic, histologic, molecular changes) and dysfunction (behavioral changes), as well as on central nervous system changes.

b) FIV(siRNA) development and administration

202. A pseudotyped lentivirus can be used to drive expression of small inhibitory RNA (siRNA) species mouse joints where interleukin-1 driven inflammation has been initiated. These

short double stranded RNAs with sizes of 19-21 base pairs can efficiently mediate gene silencing in mammalian cells by guiding sequence-specific degradation of target mRNA sequences both in vitro and in vivo (Hannon, 2002)., FIV based vectors that contain an RNA polymerase III promoter, which drives expression of single stranded RNA can be used to deliver inhibitory RNA species in vivo. These RNA species contain stem-loop structures that form short hairpin RNAs (shRNA) after intracellular processing (System Biosciences; Paddison et al., 2004). Approaches such as these have been used in a wide variety of in vivo systems (Tiscornia et al., 2003; Rubinson et al., 2003) including localized siliencing of specific gene expression in brain (Hommel et al., 2003). siRNAs are disclosed herein that effectively mediate gene silencing of a number of key molecules involved in the production of the lipid mediate Prostaglandin E_2. This includes, for example, COX-1 and -2, and the cytosolic and type-1 membrane associated prostaglandin E2 synthases (PGES). Sequences coding for shRNA's can be sub-cloned into FIV cloning vectors containing green fluorescent protein (GFP) reporter genes. After successful testing of siRNA activity upon transfection of cells in culture, VSV-G pseudotyped viral particles can be packaged by simultaneous expression of the lentiviral expression/cloning vector and packaging vectors in 293T cells. These viral particles can be infection competent but replication incompetent and can be tested and tittered in vitro before being injected into inflamed joints.

c) Pharmacologic anti-inflammatory regimen

203. A COX-2 selective inhibitor (NS-398; Kyrkanides et al., 2002), a COX-1 inhibitor SC-560 (64 ppm in chow) or a mixed inhibitor (ibuprofen at 375 ppm in chow) can be administered to mice via chow. This route of administration simplifies long-term treatment (weeks-months) and the oral doses required for specifically inhibiting these enzymes in vivo have been previously determined (Jantzen et al., 2002; Mueller-Decker et al., 2002).

d) Results & alternatives

204. Anti-inflammatory therapy can attenuate nociception. However, NSAIDs could influence inflammation by COX-independent mechanisms. For example, some NSAIDs are ligands for peroxisome proliferator-activated receptor PPAR-γ and could exert anti-inflammatory effects by this mechanism. In addition, several NSAIDS have been shown to downregulate pro-inflammatory NF-κB activity by inhibiting IκB kinase. Another point of potential importance is that drugs targeting COX isoforms could lead to concomitant upregulation of the parallel 5-lipoxygenase pathway. Recent studies on the effectiveness of dual inhibitors of cyclooxygenase and 5-lipoxygenase (ML3000) suggest that simultaneous inhibition may be required to obtain

adequate levels of anti-inflammatory action. In fact, such drugs are currently in Phase III clinical trials in Europe. Such avenues can also be used herein.

205. Interestingly, COX-1 and COX-2, in addition to their roles in peripheral inflammation, both appear to be involved to some degree in the central processing of pain at the level of the central nervous system. Overall, behavioral and pathological benefits are expected from NS-398 administration and COX-2 knockout mice. In some model systems COX-1 has been found to influence inflammation and pain. Thus, results with the COX-1 selective inhibitor and COX-1 knockout mice could also show benefits. Recently, in addition to COX-1 and COX-2, at least two new PGE_2 synthase isoforms have been added to the family of enzymes that result in the production of prostaglandins: the membrane-associated mPGES, which is functionally coupled to COX-2, and the cytosolic cPGES that appears to be linked to COX-1 dependent PGE_2 production. Although cellular localization may play some role, functional coupling is largely a factor of expression patterns: as with COX-2, mPGES is dramatically upregulated by proinflammatory stimuli, whereas cPGES is constitutively expressed in cell systems examined to date. In addition, COX-2 and mPGES are coordinately upregulated in a rat model of adjuvant arthritis. Therefore, mPGES could play a role in IL-1β-induced arthritis. Thus, the regulation of mPGES is also considered herein. This can be readily accomplished by employing established and routinely used methods. In fact, recent data suggests that COX-2 regulates mPGES at the transcriptional level, at least in an IL-1β induced brain inflammation model (Moore et al. 2004).

206. It is important to point out that the doses chosen have been previously shown to inhibit COX activity in long-term mouse experiments without evidence of serious toxicity. However, animals fed NSAID chow (and controls) can be weighed each week to assess possible toxic effects of drug treatment.

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H. Sequences

SEQ ID NO:1

human IL-1alpha mRNA (Accession No. NM_000575)

```
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 181 tctctggtcc ttggtagagg gctactttac tgtaacaggg ccagggtgga gagttctctc
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 361 tgtcacccca aactctgagg tgatttatgc cttaatcaag caaacttccc tcttcagaaa
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 601 ctcctagaaa cttgataagt ttcccgcgct tccctttttc taagactaca tgtttgtcat
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1681 aacttgttta ttgccacaaa gcaagactac tgggtgtgct tggcaggggg gccaccctct
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2761 ccatgagacc actgttatca aaactttctt ttctggaatg taatcaatgt ttcttctagg
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2941 aaa
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SEQ ID NO:2

Human IL-1beta mRNA (Accession No. NM_000576)

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SEQ ID NO:3

Human IL-1beta open reading frame – mature form

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SEQ ID NO:4

IL-1β, Sequence of ssIL-1β (539 BP)

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SEQ ID NO:5

Human IL1-RA mRNA (Accession No. NM_173842)

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SEQ ID NO:6

Human IL1-RA open reading frame

```
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```

SEQ ID NO:7 IL-1RA, Sequence of hIL-1RA (534 BP, note silent T→C mutation at position 390)

```
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SEQ ID NO:8 human IL-1R1 mRNA (Accession No. NM_000877)

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2581 ccatacacat ccccagccag aagttagtgt ccgaagaccg aattttattt tacagagctt
2641 gaaaactcac ttcaatgaac aaagggattc tccaggattc caaagttttg aagtcatctt
2701 agctttccac aggagggaga gaacttaaaa aagcaacagt agcagggaat tgatccactt
2761 cttaatgctt tcctccctgg catgaccatc ctgtcctttg ttattatcct gcattttacg
2821 tctttggagg aacagctccc tagtggcttc ctccgtctgc aatgtccctt gcacagccca
2881 cacatgaacc atccttccca tgatgccgct cttctgtcat cccgctcctg ctgaaacacc
2941 tcccaggggc tccacctgtt caggagctga agcccatgct ttcccaccag catgtcactc
3001 ccagaccacc tccctgccct gtcctccagc ttcccctcgc tgtcctgctg tgtgaattcc
3061 caggttggcc tggtggccat gtcgcctgcc cccagcactc ctctgtctct gctcttgcct
3121 cgacccttcc tcctcctttg cctaggaggc cttctcgcat tttctctagc tgatcagaat
3181 tttaccaaaa ttcagaacat cctccaattc cacagtctct gggagacttt ccctaagagg
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3361 ggctgtctct gtccctcact gccttccagg agcaatttgc acatgtaaca tagatttatg
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3481 attttatata tagagaaagt gacctatttt ttaaaaaaat cacactctaa gttctattga
3541 acctaggact tgagcctcca tttctggctt ctagtctggt gttctgagta cttgatttca
3601 ggtcaataac ggtccccccc cactccacac tggcacgttt gtgagaagaa atgacatttt
3661 gctaggaagt gaccgagtct aggaatgctt ttattcaaga caccaaattc caaacttcta
3721 aatgttggaa ttttcaaaaa ttgtgtttag attttatgaa aaactcttct actttcatct
3781 attctttccc tagaggcaaa catttcttaa aatgtttcat tttcattaaa aatgaaagcc
3841 aaatttatat gccaccgatt gcaggacaca agcacagttt taagagttgt atgaacatgg
3901 agaggacttt tggtttttat atttctcgta tttaatatgg gtgaacacca acttttattt
3961 ggaataataa ttttcctcct aaacaaaaac acattgagtt taagtctctg actcttgcct
4021 ttccacctgc tttctcctgg gcccgctttg cctgcttgaa ggaacagtgc tgttctggag
4081 ctgctgttcc aacagacagg gcctagcttt catttgacac acagactaca gccagaagcc
4141 catggagcag ggatgtcacg tcttgaaaag cctattagat gttttacaaa tttaattttg
4201 cagattattt tagtctgtca tccagaaaat gtgtcagcat gcatagtgct aagaaagcaa
4261 gccaatttgg aaacttaggt tagtgacaaa attggccaga gagtgggggt gatgatgacc
4321 aagaattaca agtagaatgg cagctggaat ttaaggaggg acaagaatca atggataagc
4381 gtgggtggag gaagatccaa acagaaaagt gcaaagttat tccccatctt ccaagggttg

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4441 aattctggag gaagaagaca cattcctagt tccccgtgaa cttcctttga cttattgtcc
4501 ccactaaaac aaaacaaaaa acttttaatg ccttccacat taattagatt ttcttgcagt
4561 ttttttatgg catttttta aagatgccct aagtgttgaa gaagagtttg caaatgcaac
4621 aaaatattta attaccggtt gttaaaactg gtttagcaca atttatattt tccctctctt
4681 gcctttctta tttgcaataa aaggtattga gccatttttt aaatgacatt tttgataaat
4741 tatgtttgta ctagttgatg aaggagtttt ttttaacctg tttatataat tttgcagcag
4801 aagccaaatt ttttgtatat taaagcacca aattcatgta cagcatgcat cacggatcaa
4861 tagactgtac ttattttcca ataaaatttt caaactttgt actgttaaa
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SEQ ID NO:9 human IL-1R2 mRNA (Accession No. NM_173343)

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   1 gggatgggag atactgttgt ggtcacctct ggaaaataca ttctgctact cttaaaaact
  61 agtgacgctc atacaaatca acagaaagag cttctgaagg aagactttaa agctgcttct
 121 gccacgtgct gctgggtctc agtcctccac ttcccgtgtc ctctggaagt tgtcaggagc
 181 aatgttgcgc ttgtacgtgt tggtaatggg agtttctgcc ttcacccttc agcctgcggc
 241 acacacaggg gctgccagaa gctgccggtt tcgtgggagg cattacaagc gggagttcag
 301 gctggaaggg gagcctgtag ccctgaggtg cccccaggtg ccctactggt tgtgggcctc
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 421 agaagaagag acacggatgt gggcccagga cggtgctctg tggcttctgc cagccttgca
 481 ggaggactct ggcacctacg tctgcactac tagaaatgct tcttactgtg acaaaatgtc
 541 cattgagctc agagtttttg agaatacaga tgctttcctg ccgttcatct catacccgca
 601 aattttaacc ttgtcaacct ctggggtatt agtatgccct gacctgagtg aattcacccg
 661 tgacaaaact gacgtgaaga ttcaatggta caaggattct cttcttttgg ataaagacaa
 721 tgagaaattt ctaagtgtga gggggaccac tcacttactc gtacacgatg tggccctgga
 781 agatgctggc tattaccgct gtgtcctgac atttgcccat gaaggccagc aatacaacat
 841 cactaggagt attgagctac gcatcaagaa aaaaaaagaa gagaccattc ctgtgatcat
 901 ttccccccctc aagaccatat cagcttctct ggggtcaaga ctgacaatcc cgtgtaaggt
 961 gtttctggga accggcacac ccttaaccac catgctgtgg tggacggcca atgacaccca
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1081 aaataatgag aactacattg aagtgccatt gatttttgat cctgtcacaa gagaggattt
1141 gcacatggat tttaaatgtg ttgtccataa taccctgagt tttcagacac tacgcaccac
1201 agtcaaggaa gcctcctcca cgttctcctg gggcattgtg ctggccccac tttcactggc
1261 cttcttggtt ttgggggggaa tatggatgca cagacggtgc aaacacagaa ctggaaaagc
1321 agatggtctg actgtgctat ggcctcatca tcaagacttt caatcctatc ccaagtgaaa
1381 taaatggaat gaaataattc aaacacaaaa aaaaaaaaaa aaaaaaaaaa aaaaaa
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SEQ ID NO:10 human COX1 mRNA (Accession No. M59979)

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   1 gcgccatgag ccggagtctc ttgctccggt tcttgctgtt cctgctcctg ctcccgccgc
  61 tccccgtcct gctcgcggac ccaggggcgc ccacgccagt gaatccctgt tgttactatc
 121 catgccagca ccagggcatc tgtgtccgct tcggccttga ccgctaccag tgtgactgca
 181 cccgcacggg ctattccggc cccaactgca ccatccctgg cctgtggacc tggctccgga
 241 attcactgcg gcccagcccc tctttcaccc acttcctgct cactcacggg cgctggttct
 301 gggagtttgt caatgccacc ttcatccgag agatgctcat gcgcctggta ctcacagtgc
 361 gctccaacct tatccccagt ccccccacct acaactcagc acatgactac atcagctggg
 421 agtctttctc caacgtgagc tattacactc gtattctgcc ctctgtgcct aaagattgcc
 481 ccacacccat gggaaccaaa gggaagaagc agttgccaga tgcccagctc ctggcccgcc
 541 gcttcctgct caggaggaag ttcatacctg acccccaagg caccaacctc atgtttgcct
 601 tctttgcaca acacttcacc caccagttct tcaaaacttc tggcaagatg ggtcctggct
 661 tcaccaaggc cttgggccat ggggtagacc tcggccacat ttatggagac aatctggagc
 721 gtcagtatca actgcggctc tttaaggatg ggaaactcaa gtaccaggtg ctggatggag
 781 aaatgtaccc gccctcggta gaagaggcgc ctgtgttgat gcactacccc cgaggcatcc
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 901 tgtatgccac gctctggcta cgtgagcaca accgtgtgtg tgacctgctg aaggctgagc
 961 accccacctg gggcgatgag cagcttttcc agacgacccg cctcatcctc ataggggaga
1021 ccatcaagat tgtcatcgag gagtacgtgc agcagctgag tggctatttc ctgcagctga
1081 aatttgaccc agagctgctg ttcggtgtcc agttccaata ccgcaaccgc attgccatgg
1141 agttcaacca tctctaccac tggcaccccc tcatgcctga ctccttcaag gtgggctccc
1201 aggagtacag ctacgagcag ttcttgttca acacctccat gttggtggac tatggggttg
1261 aggccctggt ggatgccttc tctcgccaga ttgctggccg gatcggtggg ggcaggaaca
1321 tggaccacca catcctgcat gtggctgtgg atgtcatcag ggagtctcgg gagatgcggc
1381 tgcagccctt caatgagtac cgcaagaggt ttggcatgaa accctacacc tccttccagg
1441 agctcgtagg agagaaggag atggcagcag agttggagga attgtatgga gacattgatg
1501 cgttggagtt ctaccctgga ctgcttcttg aaaagtgcca tccaaactct atctttgggg
1561 agagtatgat agagattggg gctccctttt ccctcaaggg tctcctaggg aatcccatct
1621 gttctccgga gtactggaag ccgagcacat ttggcggcga ggtgggcttt aacattgtca
1681 agacggccac actgaagaag ctggtctgcc tcaacaccaa gacctgtccc tacgtttcct
1741 tccgtgtgcc ggatgccagt caggatgatg ggcctgctgt ggagcgacca tccacagagc
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1861 gctgaggcca gggctgatgg tcttaaatgc tcattttctg gtttggcatg gtgagtgttg
1921 gggttgacat ttagaacttt aagtctcacc cattatctgg aatattgtga ttctgtttat
1981 tcttccagaa tgctgaactc cttgttagcc cttcagattg ttaggagtgg ttctcatttg
2041 gtctgccaga atactgggtt cttagttgac aacctagaat gtcagatttc tggttgattt
2101 gtaacacagt cattctagga tgtggagcta ctgatgaaat ctgctagaaa gttaggggt
2161 tcttattttg cattccagaa tcttgacttt ctgattggtg attcaaagtg ttgtgttccc
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2281 atgtggattt gattcatttt cctgttcagt gagatatcat agagacggag atcctaaggt
2341 ccaacaagaa tgcattccct gaatctgtgc ctgcactgag agggcaagga agtggggtgt
2401 tcttcttggg acccccacta agaccctggt ctgaggatgt agagagaaca ggtgggctgt
2461 attcacgcca ttggttggaa gctaccagag ctctatcccc atccaggtct tgactcatgg
2521 cagctgtttc tcatgaagct aataaaattc gccc
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SEQ ID NO:11 human COX2 mRNA (Accession No. NM_000963)

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   1 caattgtcat acgacttgca gtgagcgtca ggagcacgtc caggaactcc tcagcagcgc
  61 ctccttcagc tccacagcca gacgccctca gacagcaaag cctacccccg cgccgcgccc
 121 tgcccgccgc tcggatgctc gcccgcgccc tgctgctgtg cgcggtcctg gcgctcagcc
 181 atacagcaaa tccttgctgt tcccacccat gtcaaaaccg aggtgtatgt atgagtgtgg
 241 gatttgacca gtataagtgc gattgtaccc ggacaggatt ctatggagaa aactgctcaa
 301 caccggaatt tttgacaaga ataaaattat ttctgaaacc cactccaaac acagtgcact
 361 acatacttac ccacttcaag ggattttgga acgttgtgaa taacattccc ttccttcgaa
 421 atgcaattat gagttatgtc ttgacatcca gatcacattt gattgacagt ccaccaactt
 481 acaatgctga ctatggctac aaaagctggg aagccttctc taacctctcc tattatacta
 541 gagcccttcc tcctgtgcct gatgattgcc cgactccctt gggtgtcaaa ggtaaaaagc
 601 agcttcctga ttcaaatgag attgtggaaa aattgcttct aagaagaaag ttcatccctg
 661 atccccaggg ctcaaacatg atgtttgcat tctttgccca gcacttcacg catcagtttt
 721 tcaagacaga tcataagcga gggccagctt tcaccaacgg gctgggccat ggggtggact
 781 taaatcatat ttacggtgaa actctggcta gacagcgtaa actgcgcctt ttcaaggatg
 841 gaaaaatgaa atatcagata attgatggag agatgtatcc tcccacagtc aaagatactc
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1021 acagagtatg cgatgtgctt aaacaggagc atcctgaatg gggtgatgag cagttgttcc
1081 agacaagcag gctaatactg ataggagaga ctattaagat tgtgattgaa gattatgtgc
1141 aacacttgag tggctatcac ttcaaactga aatttgaccc agaactactt ttcaacaaac
1201 aattccagta ccaaaatcgt attgctgctg aatttaacac cctctatcac tggcatcccc
1261 ttctgcctga caccttcaa attcatgacc agaaatacaa ctatcaacag tttatctaca
1321 acaactctat attgctggaa catggaatta cccagtttgt tgaatcattc accaggcaaa
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1441 cttccattga ccagagcagg cagatgaaat accagtcttt taatgagtac cgcaaacgct
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1621 aaaagcctcg gccagatgcc atctttggtg aaaccatggt agaagttgga gcaccattct
1681 ccttgaaagg acttatgggt aatgttatat gttctcctgc ctactggaag ccaagcactt
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1921 tactaaaaga acgttcgact gaactgtaga agtctaatga tcatatttat ttatttatat
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2041 aacatcttct gtaacagaag tcagtactcc tgttgcggag aaaggagtca tacttgtgaa
2101 gacttttatg tcactactct aaagattttg ctgttgctgt taagtttgga aaacagtttt
2161 tattctgttt tataaaccag agagaaatga gttttgacgt ctttttactt gaatttcaac
2221 ttatattata agaacgaaag taaagatgtt tgaatactta aacactatca caagatggca
2281 aaatgctgaa agtttttaca ctgtcgatgt ttccaatgca tcttccatga tgcattagaa
2341 gtaactaatg tttgaaattt taaagtactt ttggttattt ttctgtcatc aaacaaaaac
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2521 acctgtaaaa gcttgtttga tttcttaaag ttattaaact tgtacatata ccaaaaagaa
2581 gctgtcttgg atttaaatct gtaaaatcag atgaaatttt actacaattg cttgttaaaa
2641 tattttataa gtgatgttcc tttttcacca agagtataaa ccttttttagt gtgactgtta
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2761 tctcaaaata agaatatttt gttgagatat tccagaattt gtttatatgg ctggtaacat
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2881 caaattattg ttcaaattta ggtttaaact tttgaagcaa actttttttt atccttgtgc
2941 actgcaggcc tggtactcag attttgctat gaggttaatg aagtaccaag ctgtgcttga
3001 ataacgatat gttttctcag attttctgtt gtacagttta atttagcagt ccatatcaca
3061 ttgcaaaagt agcaatgacc tcataaaata cctcttcaaa atgcttaaat tcatttcaca
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3121 cattaatttt atcccagccc tgaagccaat tcagtaggtg cattggaatc aagcctggct
3181 acctgcatgc tgttcctttt cttttcttct tttagccatt ttgctaagag acacagtctt
3241 ctcatcactt cgtttctcct attttgtttt actagtttta agatcagagt tcactttctt
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3361 aggactgcta tttagctcct cttaagaaga ttaaaagaga aaaaaaaagg ccctttaaa
3421 aatagtatac acttatttta agtgaaaagc agagaatttt atttatagct aattttagct
3481 atctgtaacc aagatggatg caaagaggct agtgcctcag agagaactgt acggggtttg
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3781 ttttttgtta tgtcacaatc agtatatttt ctttggggtt acctctctga atattatgta
3841 aacaatccaa agaaatgatt gtattaagat ttgtgaataa atttttagaa atctgattgg
3901 catattgaga tatttaaggt tgaatgtttg tccttaggat aggcctatgt gctagcccac
3961 aaagaatatt gtctcattag cctgaatgtg ccataagact gacctttaa aatgttttga
4021 gggatctgtg gatgcttcgt taatttgttc agccacaatt tattgagaaa atattctgtg
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4261 agaaatagtc aatatgcttg tataaaacac tgttcactgt ttttttaaa aaaaaaactt
4321 gatttgttat taacattgat ctgctgacaa aacctgggaa tttgggttgt gtatgcgaat
4381 gtttcagtgc ctcagacaaa tgtgtattta acttatgtaa aagataagtc tggaaataaa
4441 tgtctgttta tttttgtact attta
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SEQ ID NO:12 human mPGES mRNA (Accession No. NM_004878)

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   1 gctgctcctc tgtcgagctg atcacaccca cagttgagct gcgctggcca gagatgcctg
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 121 tggtcatcaa gatgtacgtg gtggccatca tcacgggcca agtgaggctg cggaagaagg
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 361 tcctggtctt cctcgtgggc cgtgtggcac acaccgtggc ctacctgggg aagctgcggg
 421 cacccatccg ctccgtgacc tacaccctgg cccagctccc ctgcgcctcc atggctctgc
 481 agatcctctg ggaagcggcc cgccacctgt gaccagcagc tgatgcctcc ttggccacca
 541 gaccatgggc caagagccgc cgtggctata cctggggact tgatgttcct tccagattgt
 601 ggtgggccct gagtcctggt ttcctggcag cctgctgcgc gtgtgggtct ctgggcacag
 661 tgggcctgtg tgtgtgcccg tgtgtgtgta tgtgtgtgtg tatgtttctt agccccttgg
 721 attcctgcac gaagtggctg atgggaacca tttcaagaca gattgtgaag attgatagaa
 781 aatccttcag ctaaagtaac agagcatcaa aaacatcact ccctctccct ccctaacagt
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1261 gtgctcagcc accatctgga gttttaaaag gctcccatgt gagtccctgt gatggccagg
1321 ccaggggacc cctgccagtt ctctgtggaa gcaaggctgg ggtcttgggt tcctgtatgg
1381 tggaagctgg gtgagccaag gacagggctg gctcctctgc ccccgctgac gcttcccttg
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1501 gcccacagga agctcagcct tctcctccca aggtttgagt ccctccaaag ggcagtgggt
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1621 cttcaaggtt tggaaactgc aaatgtcccc ttgatgggga atccgtgtgt gtgtgtgtgt
1681 gtgtgtgtgt gtgtgtgtgt gtgtgtgttt tctcctagac ccgtgacctg agatgtgtga
1741 tttttagtca ttaaatggaa gtgtctgcca gctgggccca gcacctaaaa aaaaaaaaaa
1801 aaaaa
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SEQ ID NO:13 human cPGES/p23 mRNA (Accession No. L24804)

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 121 gccgcgaccg agagaaaaag cggagtcgca ccggagagaa gtcgactccc tagcagcagc
 181 cgccgccaga gagcccgccc accagttcgc ccgtcccccc gccccgttca caatgcagcc
 241 tgcttctgca aagtggtacg atcgaaggga ctatgtcttc attgaatttt gtgttgaaga
 301 cagtaaggat gttaatgtaa attttgaaaa atccaaactt acattcagtt gtctcggagg
 361 aagtgataat tttaagcatt taaatgaaat tgatcttttt cactgtattg atccaaatga
 421 ttccaagcat aaaagaacgg acagatcaat tttatgttgt ttacgaaaag gagaatctgg
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481 ccagtcatgg ccaaggttaa caaaagaaag ggcaaagctt aattggctta gtgtcgactt
541 caataattgg aaagactggg aagatgattc agatgaagac atgtctaatt ttgatcgttt
601 ctctgagatg atgaacaaca tgggtggtga tgaggatgta gatttaccag aagtagatgg
661 agcagatgat gattcacaag acagtgatga tgaaaaaatg ccagatctgg agtaaggaat
721 attgtcatca cctggatttt gagaaagaaa aataacttct ctgcaagatt tcataattga
781 ga
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SEQ ID NO:14 Secretion Signal sequence of human IL-1ra: (75 bp)

```
atggaaatctgcagaggcctccgcagtcacctaatcactctcctcctcttcctgttccattcagagacgatctgc
```

SEQ ID NO:15 CMV sequence

```
Cgatgtacgggccagatatacgcgttgacattgattattgactagttattaatagtaatcaattacggggtcattag
ttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccc
cgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtgga
ctatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatg
acggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgta
ttagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacgggg
atttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtc
gtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctctctgg
ctaactagagaacccactgcttactggcttatcgaaatt
```

SEQ ID NO:16 Chicken Beta Actin promoter

```
tcgaggtgag ccccacgttc tgcttcactc tccccatctc ccccccctcc ccacccccaa      60
ttttgtattt atttatttttt taattatttt gtgcagcgat gggggcgggg ggggggggg      120
cgcgcgccag gcggggcggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg      180
gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg      240
cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag tcgctgcgtt gccttcgccc      300
cgtgccccgc tccgcgccgc ctcgcgccgc ccgccccggc tctgactgac cgcgttactc      360
ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg cttggtttaa      420
tgacggctcg tttcttttct gtggctgcgt gaaagcctta aagggctccg ggagggccct      480
ttgtgcgggg gggagcggct cgggggggtgc gtgcgtgtgt gtgtgcgtgg ggagcgccgc      540
gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg gctttgtgcg      600
ctccgcgtgt gcgcgagggg agcgcggccg ggggcggtgc cccgcggtgc gggggggctg      660
cgaggggaac aaaggctgcg tgcggggtgt gtgcgtgggg gggtgagcag ggggtgtggg      720
cgcggcggtc gggctgtaac ccccccctgc acccccctcc ccgagttgct gcgcacggcc      780
cggcttcggg tgcggggctc cgtgcggggc gtggcgcggg gctcgccgtg ccgggcgggg      840
ggtggcggca ggtgggggtg ccgggcgggg cggggccgcc tcgggccggg gagggctcgg      900
gggaggggcg cggcggcccc ggagcgccgg cggctgtcga ggcgcggcga gccgcagcca      960
ttgcctttta tggtaatcgt gcgagagggc gcagggactt cctttgtccc aaatctggcg     1020
gagccgaaat ctgggaggcg ccgccgcacc ccctctagcg ggcgcgggcg aagcggtgcg     1080
gcgccggcag gaaggaaatg ggcggggagg gccttcgtgc gtcgccgcgc cgccgtcccc     1140
ttctccatct ccagcctcgg ggctgccgca gggggacggc tgccttcggg ggggacgggg     1200
cagggcgggg ttcggcttct ggcgttgtac cggcggggtt tatatcttcc cttctctgtt     1260
cctccgcagc cagccatg                                                  1278
```

SEQ ID NO:17 E06566 Accession # Promoter gene of human beta-actin gene

```
cccgggccca gcaccccaag gcggccaacg ccaaaactct ccctcctcct cttcctcaat      60
ctcgctctcg ctctttttt ttttcgcaaa aggaggggag agggggtaaa aaaatgctgc      120
actgtgcggc gaagccggtg agtgagcggc gcggggccaa tcagcgtgcg ccgttccgaa      180
agttgccttt tatggctcga gcggccgcgg cggcgcccta taaaacccag cggcgcgacg      240
cgccaccacc gccgagaccg cgtccgcccc gcgagcacag agcctcgcct ttgccgatcc      300
gccgcccgtc cacacccgcc gccaggtaag cccggccagc cgaccggggc atgcggccgc      360
ggcccccttcg cccgtgcaga gccgccgtct gggccgcagc gggggggcgca tgggggggga     420
accggaccgc cgtgggggggc gcgggagaag cccctgggcc tccggagatg ggggacaccc      480
cacgccagtt cggaggcgcg aggccgcgct cgggaggcgc gctccgggggg tgccgctctc      540
ggggcggggg caaccggcgg ggtctttgtc tgagccgggc tcttgccaat ggggatcgca      600
gggtgggcgc ggcgtagccc ccgccaggcc cggtgggggc tggggcgcca ttgccggtgc      660
gcgctggtcc tttgggcgct aactgcgtgc gcgctgggaa ttggcgctaa ttgcgcgtgc      720
gcgctgggac tcaaggcgct aattgcgcgt gcgttctggg gcccggggtg ccgcggcctg      780
ggctggggcg aaggcgggct cggccggaag gggtggggtc gccgcggctc ccgggcgctt      840
gcgcgcactt cctgcccgag ccgctggccg cccgagggtg tggccgctgc gtgcgcgcgc      900
gccgacccgg cgctgtttga accgggcgga ggcggggctg gcgcccggtt gggagggggt      960
tggggcctgg cttcctgccg cgcgccgcgg ggacgcctcc gaccagtgtt tgccttttat     1020
```

```
ggtaataacg cggccggccc ggcttccttt gtccccaatc tgggcgcgcg ccggcgcccc   1080
ctggcggcct aaggactcgg cgcgccggaa gtggccaggg cgggggcgac ttcggctcac   1140
agcgcgcccg gctattctcg cagctcacca tggatg                             1176
```

SEQ ID NO:18 CMV-Beta actin promoter

```
gaattcggta ccctagttat taatagtaat caattacggg gtcattagtt catagcccat     60
atatggagtt ccgcgttaca taacttacgg taaatggccc gcctggctga ccgcccaacg    120
acccccgccc attgacgtca ataatgacgt atgttcccat agtaacgcca atagggactt    180
tccattgacg tcaatgggtg gactatttac ggtaaactgc ccacttggca gtacatcaag    240
tgtatcatat gccaagtacg ccccctattg acgtcaatga cggtaaatgg cccgcctggc    300
attatgccca gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag    360
tcatcgctat taccatggtc gaggtgagcc ccacgttctg cttcactctc cccatctccc    420
ccccctcccc acccccaatt ttgtatttat ttatttttta attattttgt gcagcgatgg    480
gggcgggggg gggggggggg cgcgcgccag gcggggcggg gcggggcgag gggcggggcg    540
gggcgaggcg gagaggtgcg gcggcagcca atcagagcgg cgcgctccga aagtttcctt    600
ttatggcgag gcggcggcgg cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag    660
tcgctgcgac gctgccttcg ccccgtgccc cgctccgccg ccgcctcgcg ccgcccgccc    720
cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg    780
ggctgtaatt agcgcttggt ttaatgacgg cttgtttctt ttctgtggct gcgtgaaagc    840
cttgaggggc tccgggaggg ccctttgtgc gggggggagc ggctcggggg gtgcgtgcgt    900
gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc    960
gggcgcggcg cggggctttg tgcgctccgc agtgtgcgcg aggggagcgc ggccgggggc   1020
ggtgccccgc ggtgcggggg gggctgcgag gggaacaaag gctgcgtgcg gggtgtgtgc   1080
gtgggggggt gagcaggggg tgtgggcgcg gcggtcgggc tgtaaccccc ccctgcaccc   1140
ccctccccga gttgctgagc acggcccggc ttcgggtgcg gggctccgta cggggcgtgg   1200
cgcggggctc gccgtgccgg gcgggggggtg gcggcaggtg ggggtgccgg gcgggggcggg  1260
gccgcctcgg gccggggagg gctcgggggga ggggcgcggc ggcccccgga gcgccggcgg   1320
ctgtcgaggc gcggcgagcc gcagccattg ccttttatgg taatcgtgcg agagggcgca   1380
gggacttcct ttgtcccaaa tctgtgcgga gccgaaatct gggaggcgcc gccgcacccc   1440
ctctagcggg cgcggggcga agcggtgcgg cgccggcagg aaggaaatgg gcggggaggg   1500
ccttcgtgcg tcgccgcgcc gccgtcccct tctccctctc cagcctcggg gctgtccgcg   1560
gggggacggc tgccttcggg ggggacgggg cagggcgggg ttcggcttct ggcgtgtgac   1620
cggcggctct agagcctctg ctaaccatgt tcatgccttc ttctttttcc tacagctcct   1680
gggcaacgtg ctggttattg tgctgtctca tcattttggc aaagaattc               1729
```

SEQ ID NO:19 Chicken beta-actin with CMV enhancer elements promoter sequence

```
cgatgtacgggccagatatacgcgttgacattgattattgactagttattaatagtaatcaattacggggtcattag
ttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccc
cgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtgga
ctatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatg
acggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgta
ttagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacgggg
atttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtc
gtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctctctgg
ctaactagagaacccactgcttactggcttatcgaaatt
```

SEQ ID NO:20 Fusion promoter-CMV portion

```
tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg     60
cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt    120
gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca    180
atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc    240
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta    300
catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac    360
catggt                                                              366
```

SEQ ID NO:21 Fusion promoter – beta actin portion

```
ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc gggggggggg     60
gggggcgcgc cgccaggcgg ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga    120
ggtgcggcgg cagccaatca gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg    180
cggcggcggc ggccctataa aaagcgaagc gcgcggcggg cgggagtcgc tgcgacgctg    240
ccttcgcccc gtgccccgct ccgccgccgc ctcgcgccgc ccgccccggc tctgactgac    300
cgcgttactc ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg    360
cttggtttaa tgacggcttg tttcttttct gtggctgcgt gaaagccttg aggggctccg    420
```

ggagggccct ttgtgcgggg gggagcggct cgggggggtgc gtgcgtgtgt gtgtgcgtgg 480
ggagcgccgc gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg 540
gctttgtgcg ctccgcagtg tgcgcgaggg gagcgcggcc gggggcggtg ccccgcggtg 600
cgggggggggc tgcgagggga acaaaggctg cgtgcggggt gtgtgcgtgg gggggtgagc 660
agggggtgtg ggcgcggcgg tcgggctgta acccccccct gcacccccct ccccgagttg 720
ctgagcacgg cccggcttcg ggtgcggggc tccgtacggg gcgtggcgcg gggctcgccg 780
tgccgggcgg ggggtggcgg caggtggggg tgccgggcgg ggcggggccg cctcgggccg 840
gggagggctc gggggagggg cgcggcggcc cccggagcgc cggcggctgt cgaggcgcgg 900
cgagccgcag ccattgcctt ttatggtaat cgtgcgagag ggcgcaggga cttcctttgt 960
cccaaatctg tgcggagccg aaatctggga ggcgccgccg cacccccctct agcgggcgcg 1020
gggcgaagcg gtgcggcgcc ggcaggaagg aaatgggcgg ggagggcctt cgtgcgtcgc 1080
cgcgccgccg tccccttctc cctctccagc ctcggggctg tccgcggggg gacggctgcc 1140
ttcgggggggg acggggcagg gcggggttcg gcttctggcg tgtgaccggc ggctctagag 1200
cctctgctaa ccatgttcat gccttcttct ttttcctaca gctcctgggc aacgtgctgg 1260
ttattgtgct gtctcatcat tttggcaaag aattc 1295

SEQ ID NO:22 Accession # BD136067. promoter element for sustained gene expression from CMV promoter

gtattagtca tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga 60
tagcggtttg actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg 120
ttttggcacc aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg 180
caaatgggcg gtaggcgtgt acggtgggag gtctatataa gcagagctc 229

SEQ IDNO:23 BD136066 Accession # promoter element for sustained gene expression from CMV promoter

tggcattatg cccagtacat gaccttatgg gactttccta cttggcagta catctacgta 60
ttagtcatcg ctattaccat ggtgatgcgg ttttggcagt acatcaatgg gcgtggatag 120
cggtttgact cacggggatt tccaagtctc caccccattg acgtcaatgg gagtttgttt 180
tggcaccaaa atcaacggga ctttccaaaa tgtcgtaaca actccgcccc attgacgcaa 240
atgggcggta ggcgtgtacg gtgggaggtc tatataagca g 281

SEQ ID NO:24 BD136065 Accession # promoter element for sustained gene expression from CMV promoter

attatgccca gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag 60
tcatcgctat taccatggtg atgcggtttt ggcagtacat caatgggcgt ggatagcggt 120
ttgactcacg gggatttcca agtctccacc ccattgacgt caatgggagt ttgttttggc 180
accaaaatca acgggacttt ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg 240
gcggtaggcg tgtacggtgg gaggtctata taagcagagc tc 282

SEQ ID NO:25 BD136064 Accession # promoter element for sustained gene expression from CMV promoter

ttgcgttaca taacttacgg taaatggccc gcctggctga ccgcccaacg acccccgccc 60
attgacgtca ataatgacgt atgttcccat agtaacgcca tagggactt tccattgacg 120
tcaatgggtg gactatttac ggtaaactgc ccacttggca gtacatcaag tgtatcatat 180
gccaagtacg ccccctattg acgtcaatga cggtaaatgg cccgcctggc attatgccca 240
gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag tcatcgctat 300
taccatggtg atgcggtttt ggcagtacat caatgggcgt ggatagcggt ttgactcacg 360
gggatttcca agtctccacc ccattgacgt caatgggagt ttgttttggc accaaaatca 420
acgggacttt ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg gcggtaggcg 480
tgtacggtgg gaggtctata taagcagagc tc 512

SED ID NO:26 L77202 Accession # Murine Cytomegalovirus early (E1) gene, promoter region

tcggcgaagc ctcgcgcggc cggccaggac gaggagcgcc actaggttga acatccgcac 60
gagccgccgg gccaggtctc ggacgggctc tcgagactcg atctcgtgca tgtcggcggt 120
ccgcggtgag gttatagacc atctgctagg cgggtccggg gagacaggca cattactggc 180
ctcggcgccc agcctaggcg tgtctagagc tcgaccgcgc gtccggagcg ccattcgacc 240
ggcgggtagc gagaagaacg ccggagaccg caggttataa caacgtcatg cataaattaa 300
gaatgggc 308

SEQ ID NO:27 X03922 Accession # Human cytomegalovirus (HCMV) IE1 gene promoter region

```
ctgcagtgaa taataaaatg tgtgtttgtc cgaaatacgc gtttgagatt tctgtcccga      60
ctaaattcat gtcgcgcgat agtggtgttt atcgccgata gagatggcga tattggaaaa     120
atcgatattt gaaaatatgg catattgaaa atgtcgccga tgtgagtttc tgtgtaactg     180
atatcgccat ttttccaaaa gttgattttt gggcatacgc gatatctggc gatacgctta     240
tatcgtttac gggggatggc gatagacgcc tttggtgact tgggcgattc tgtgtgtcgc     300
aaatatcgca gtttcgatat aggtgacaga cgatatgagg ctatatcgcc gatagaggcg     360
acatcaagct ggcacatggc caatgcatat cgatctatac attgaatcaa tattggccat     420
tagccatatt attcattggt tatatagcat aaatcaatat tggctattgg ccattgcata     480
cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca ttaccgccat     540
gttgacattg attattgact agttattaat agtaatcaat tacggggtca ttagttcata     600
gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc     660
ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag     720
ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac     780
atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg     840
cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg     900
tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat gggcgtggat     960
agcggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt    1020
tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc ccattgacgc    1080
aaatgggcgg taggcgtgta cggtgggagg tctatataag cagagctcgt ttagtgaacc    1140
gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga caccgggacc    1200
gatccagcct ccgcggccgg gaacggtgca ttggaacgcg gattccccgt gccaagagtg    1260
acgtaagtac cgcctataga gtctataggc ccacccccct ggcttcttat gcatgctata    1320
ctgtttttgg cttggggtct atacacccccc gcttcctcat gttataggtg atggtatagc    1380
ttagcctata ggtgtgggtt attgaccatt attgaccact cccctattgg tgacgatact    1440
ttccattact aatccataac atggctcttt gcacaactct ctttattggc tatatgccaa    1500
tacactgtcc ttcagagact gacacggact ctgtattttt acaggatggg gtctcattta    1560
ttatttacaa attcacatat acaacaccac cgtccccagt gcccgcagtt tttattaaac    1620
ataacgtggg atctccagcg aatctcgggt acgtgttccg gacatggggc tcttctccgg    1680
tagcggcgga gcttctacat ccagccctgc tcccatcctc ccactcatgg tcctcggcag    1740
ctccttgctc ctaacagtgg aggccagact taggcacagc acgatgccca ccaccaccag    1800
tgtgcccaca aggccgtggc ggtagggtat gtgtctgaaa atgagctc                 1848
```

SEQ ID NO:28 E02198 Accession # Dna encoding 3'end region of beta-actin gene promoter

```
cttctggcgt gtgaccggcg gggtttatat cttcccttcc caagcttgg                  49
```

SEQ ID NO:29 E02197 Accession # DNA encoding 3'end region of beta-actin gene promoter

```
cttctggcgt gtgaccggcg gggtttatat cttcccttct ctgttcctcc gcagccccaa      60
gcttgg                                                                 66
```

SEQ ID NO:30 E02196 Accession # DNA encoding 3'end region of beta-actin gene promoter

```
cttctggcgt gtgaccggcg gggtttatat cttcccttct ctgttcctcc gcagccagcc      60
aagcttgg                                                               68
```

SEQID NO:31 E02195 Accession # DNA encoding 3'end region of beta-actin gene promoter

```
cttctggcgt gtgaccggcg gggtttatat cttcccttct ctgttcctcc gcagccagcc      60
atggatgat                                                              69
```

SEQ ID NO 32 E03011 Accession # DNA encoding hybrid promoter that is composed of chicken beta-actin gene promoter and rabbit beta-globin gene promoter

```
tcgaggtgag ccccacgttc tgcttcactc tccccatctc ccccccctcc ccacccccaa      60
ttttgtattt atttatttttt taattatttt gtgcagcgat gggggcgggg gggggggggg     120
```

```
cgcgcgccag gcggggcggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg     180
gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg     240
cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag tcgctgcgtt gccttcgccc     300
cgtgccccgc tccgcgccgc ctcgcgccgc ccgccccggc tctgactgac cgcgttactc     360
ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg cttggtttaa     420
tgacggctcg tttcttttct gtggctgcgt gaaagcctta aagggctccg ggagggccct     480
ttgtgcgggg gggagcggct cgggggggtgc gtgcgtgtgt gtgtgcgtgg ggagcgccgc     540
gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg gctttgtgcg     600
ctccgcgtgt gcgcgagggg agcgcggccg ggggcggtgc cccgcggtgc gggggggctg     660
cgaggggaac aaaggctgcg tgcggggtgt gtgcgtgggg gggtgagcag ggggtgtggg     720
cgcggcggtc gggctgtaac ccccccctgc acccccctcc ccgagttgct gagcacggcc     780
cggcttcggg tgcggggctc cgtgcggggc gtggcgcggg gctcgccgtg ccgggcgggg     840
ggtggcggca ggtgggggtg ccgggcgggg cggggccgcc tcgggccggg gagggctcgg     900
gggaggggcg cggcggcccc ggagcgccgg cggctgtcga ggcgcggcga gccgcagcca     960
ttgcctttta tggtaatcgt gcgagagggc gcagggactt cctttgtccc aaatctggcg    1020
gagccgaaat ctgggaggcg ccgccgcacc ccctctagcg ggcgcgggcg aagcggtgcg    1080
gcgccggcag gaaggaaatg ggcggggagg gccttcgtgc gtcgccgcgc cgccgtcccc    1140
ttctccatct ccagcctcgg ggctgccgca gggggacggc tgccttcggg ggggacgggg    1200
cagggcgggg ttcggcttct ggcgtgtgac cggcggctct agagcctctg ctaaccatgt    1260
tcatgccttc ttctttttcc tacagctcct gggcaacgtg ctggttgttg tgctgtctca    1320
tcattttggc aaagaattca agctt                                         1345
```

SEQ ID NO:33 BD015377 Accession # Baculovirus containing minimum CMV promoter

```
tcaatattgg ccattagcca tattattcat tggttatata gcataaatca atattggcta      60
ttggccattg catacgttgt atctatatca taatatgtac atttatattg gctcatgtcc     120
aatatgaccg ccatgttggc attgattatt gactagttat taatagtaat caattacggg     180
gtcattagtt catagcccat atatggagtt ccgcgttaca taacttacgg taaatggccc     240
gcctggctga ccgcccaacg acccccgccc attgacgtca ataatgacgt atgttcccat     300
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SEQ ID NO:34 Mouse GFAP promoter sequence

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SEQ ID NO:43 cPGES_3 siRNA

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SEQ ID NO:63 mPGES-1 shRNA antisense
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SEQ ID NO:64 COX-1_3 shRNA sense
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SEQ ID NO:65 COX-1_3 shRNA antisense
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SEQ ID NO:66 COX-2_1 shRNA sense
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SEQ ID NO:67 COX-2_1 shRNA antisense
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SEQ ID NO:68 Secretion Signal sequence of human IL-1ra
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SEQ ID NO:69 Human CD11b (Mac-1) gene, 5' flank (Accession No. M82856)

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SEQ ID NO:71 IL-1alpha antisense
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SEQ ID NO:73 COX-2 antisense
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SEQ ID NO:74 cPGES antisense
CCTCTTCTCGCTTCCCTCA

SEQ ID NO:75 mPGES antisense
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SEQ ID NO:76 IL-1alpha Hammerhead Ribozyme
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SEQ ID NO:77 IL-1beta Hammerhead Ribozyme
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SEQ ID NO:78 COX-1 Hammerhead Ribozyme
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SEQ ID NO:79 COX-2 Hammerhead Ribozyme
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SEQ ID NO:80 mPGES Hammerhead Ribozyme
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SEQ ID NO:81 cPGES Hammerhead Ribozyme
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VI. CLAIMS

What is claimed is:

1. A composition comprising a viral vector, wherein delivery of the vector to a cell inhibits a mediator of inflammation.
2. The composition of claim 1, wherein the vector comprises a nucleic acid operably linked to an expression control sequence and wherein the nucleic acid inhibits expression of the mediator of inflammation.
3. The composition of claim 2, wherein the nucleic acid is an siRNA.
4. The composition of claim 3, wherein the siRNA inhibits gene expression of COX-1.
5. The composition of claim 4, wherein the siRNA comprises the nucleic acid sequence SEQ ID NO:49.
6. The composition of claim 3, wherein the siRNA inhibits gene expression of COX-2.
7. The composition of claim 6, wherein the siRNA comprises the nucleic acid sequence SEQ ID NO:53.
8. The composition of claim 3, wherein the siRNA inhibits gene expression of mPGES.
9. The composition of claim 8, wherein the siRNA comprises the nucleic acid sequence SEQ ID NO:59.
10. The composition of claim 3, wherein the siRNA inhibits gene expression of cPGES.
11. The composition of claim 10, wherein the siRNA comprises the nucleic acid sequence SEQ ID NO:43.
12. The composition of claim 1, wherein the vector comprises a nucleic acid that encodes a polypeptide that inhibits the binding of the mediator of inflammation to its receptor.
13. The composition of claim 12, where the polypeptide inhibits the binding of IL-1β to an IL-1 receptor.
14. The composition of claim 12, where the polypeptide is IL-1ra.
15. The composition of claim 14, where the polypeptide is human IL-1ra.
16. The composition of claim 12, where the nucleic acid has the sequence set forth in SEQ ID NO:5.
17. The composition of claim 12, where the nucleic acid encodes a polypeptide with at least 70%, 75%, 80%, 85%, 90%, 95% identity to the sequence set forth in SEQ ID NO:38.
18. The composition of claim 17, wherein any change is a conservative change.
19. The composition of claim 12, where the nucleic acid hybridizes to SEQ ID NO:5 under stringent conditions.
20. The composition of claim 2, wherein the expression control sequence is a constitutive

promoter.

21. The composition of claim 20, wherein the promoter is a CMV promoter.

22. The composition of claim 21, wherein the CMV promoter comprises the nucleic acid sequence set forth in SEQ ID NO:15.

23. The composition of claim 20, wherein the promoter is a beta actin promoter.

24. The composition of claim 23, wherein the beta actin promoter comprises the nucleic acid sequence set forth in SEQ ID NO:16.

25. The composition of claim 2, wherein the expression control sequence is a tissue-specific promoter.

26. The composition of claim 2, wherein the expression control sequence is an inducible promoter.

27. The composition of claim 2, wherein the vector further comprises a marker sequence.

28. The composition of claim 2, wherein the vector comprises a lentivirus.

29. The composition of claim 28, wherein the vector comprises a feline immunodeficiency virus.

30. The composition of claim 28, wherein the vector comprises a human immunodeficiency virus.

31. The composition of claim 28, wherein the vector can be stably integrated for at least 3 months.

32. A composition comprising a cell, wherein the cell comprises the vector of claim 1.

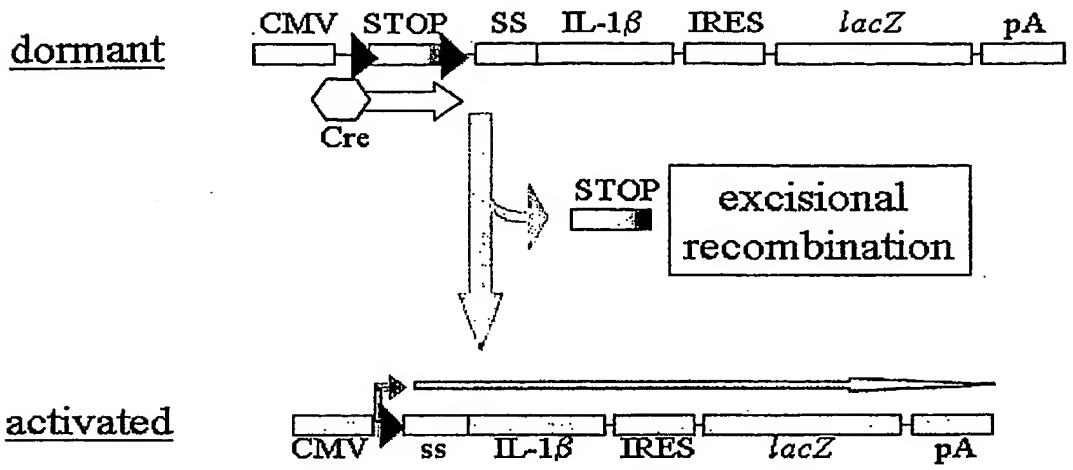
33. A method of inhibiting inflammation in a subject comprising administering to the subject the vector of claim 1.

34. A method of treating a subject with arthritis comprising administering to the subject the vector of claim 1.

35. The method of claim 34, wherein the subject has Osteoarthritis, Rheumatoid arthritis, Gout, Ankylosing spondylitis, Juvenile arthritis, Systemic lupus erythematosus (lupus), Scleroderma, or Fibromyalgia.

36. A method of treating a subject with a central nervous system disorder resulting from neuroinflammation comprising administering to the subject the vector of claim 1.

37. The method of claim 36, wherein the subject has Alzheimer's disease.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SS
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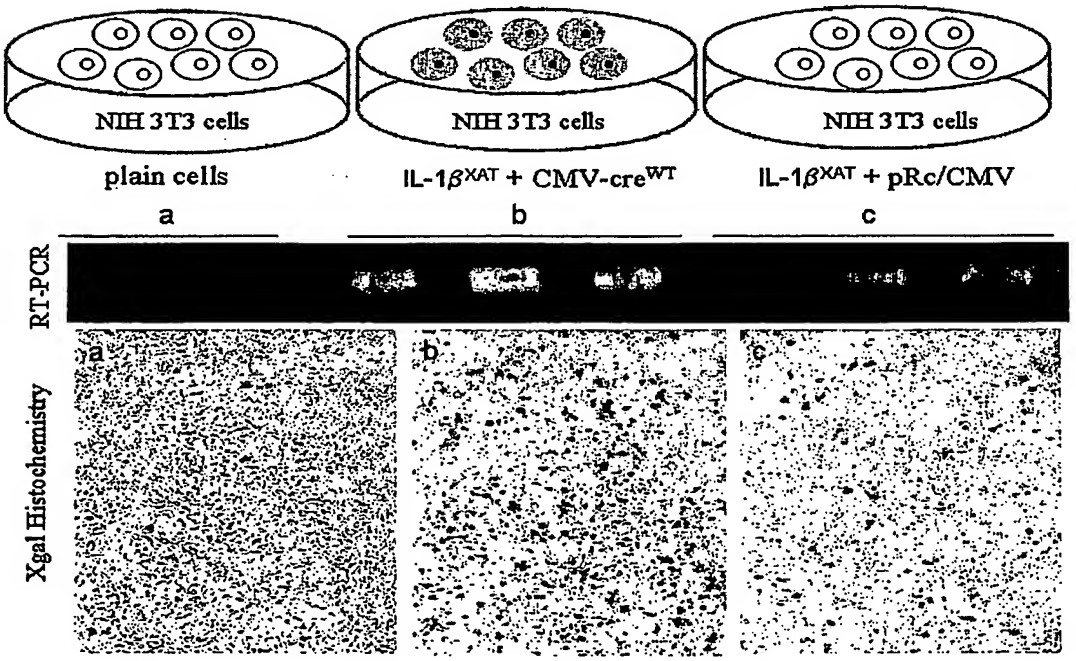
FIG. 1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plain cells
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IL-1β^XAT + pRc/CMV
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b
c
RT-PCR
Xgal Histochemistry
a
b
c

FIG. 2

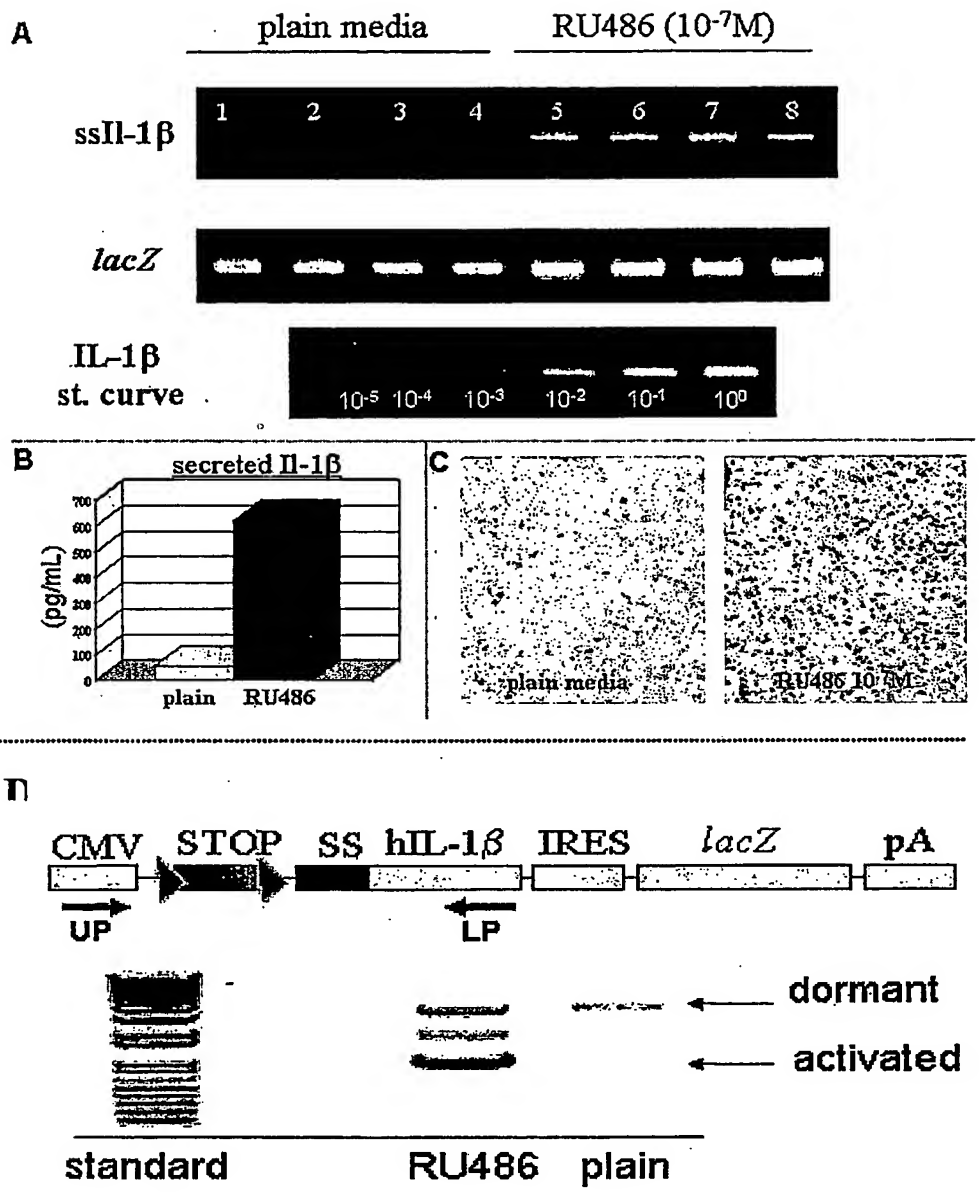
A
plain media
RU486 (10-7M)
ssIl-1β
1 2 3 4 5 6 7 8
lacZ
IL-1β
st. curve
10-5 10-4 10-3 10-2 10-1 100
B
secreted Il-1β
(pg/mL)
plain RU486
C
plain media
RU486 10-7M
D
CMV STOP SS hIL-1β IRES lacZ pA
UP
LP
dormant
activated
standard
RU486 plain

FIG. 3

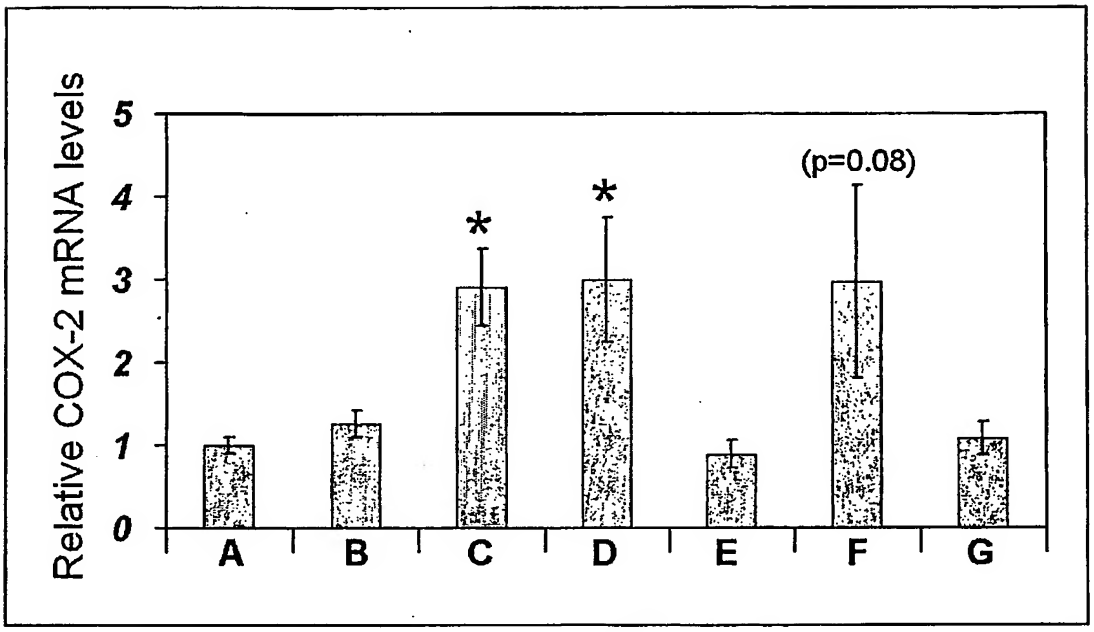

FIG. 4

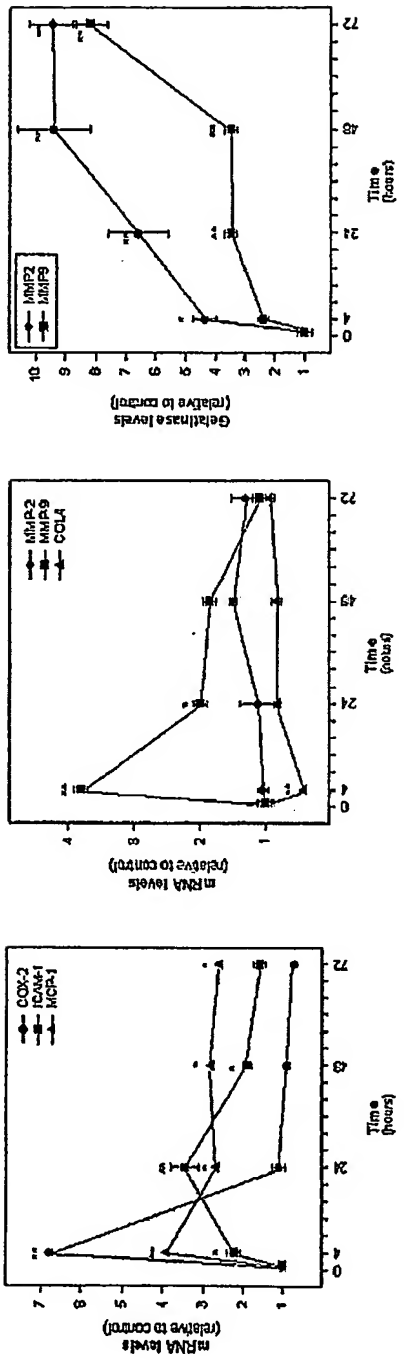
Gelatinase levels
(relative to control)
MMP2
MMP9
Time
(hours)
mRNA levels
(relative to control)
MMP-2
MMP-9
COL4
Time
(hours)
mRNA levels
(relative to control)
COX-2
ICAM-1
MCP-1
Time
(hours)

FIG. 5

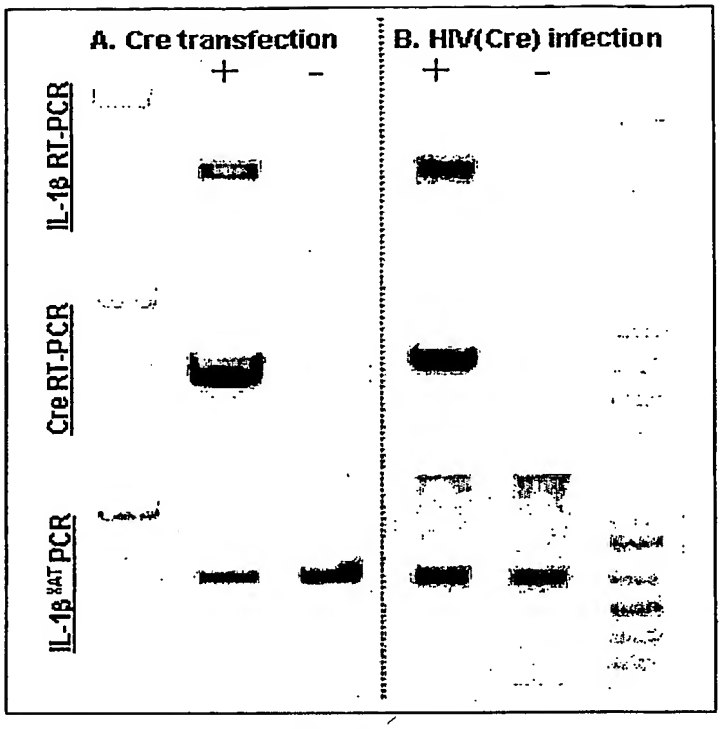
A. Cre transfection
B. HIV(Cre) infection
+
-
+
-
IL-1β RT-PCR
Cre RT-PCR
IL-1β ΔAT PCR

FIG. 6

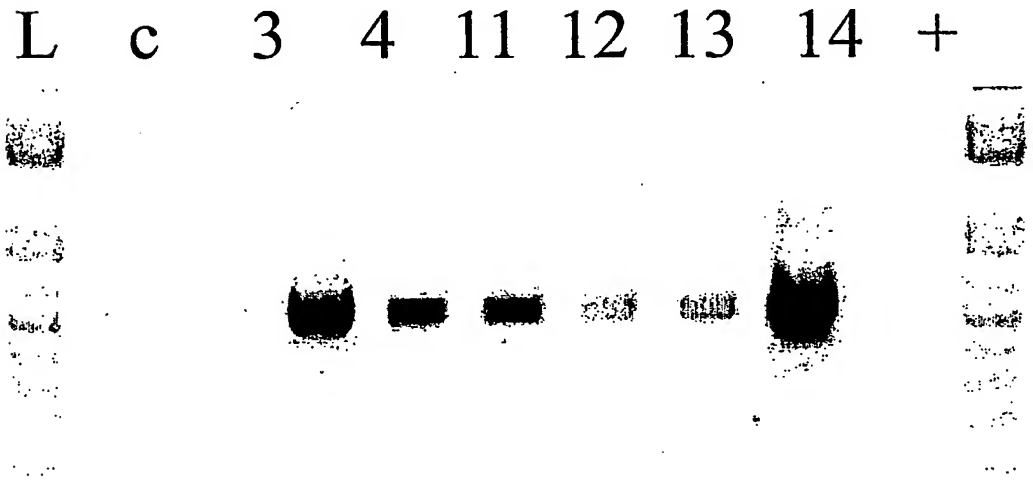
L c 3 4 11 12 13 14 +

FIG. 7

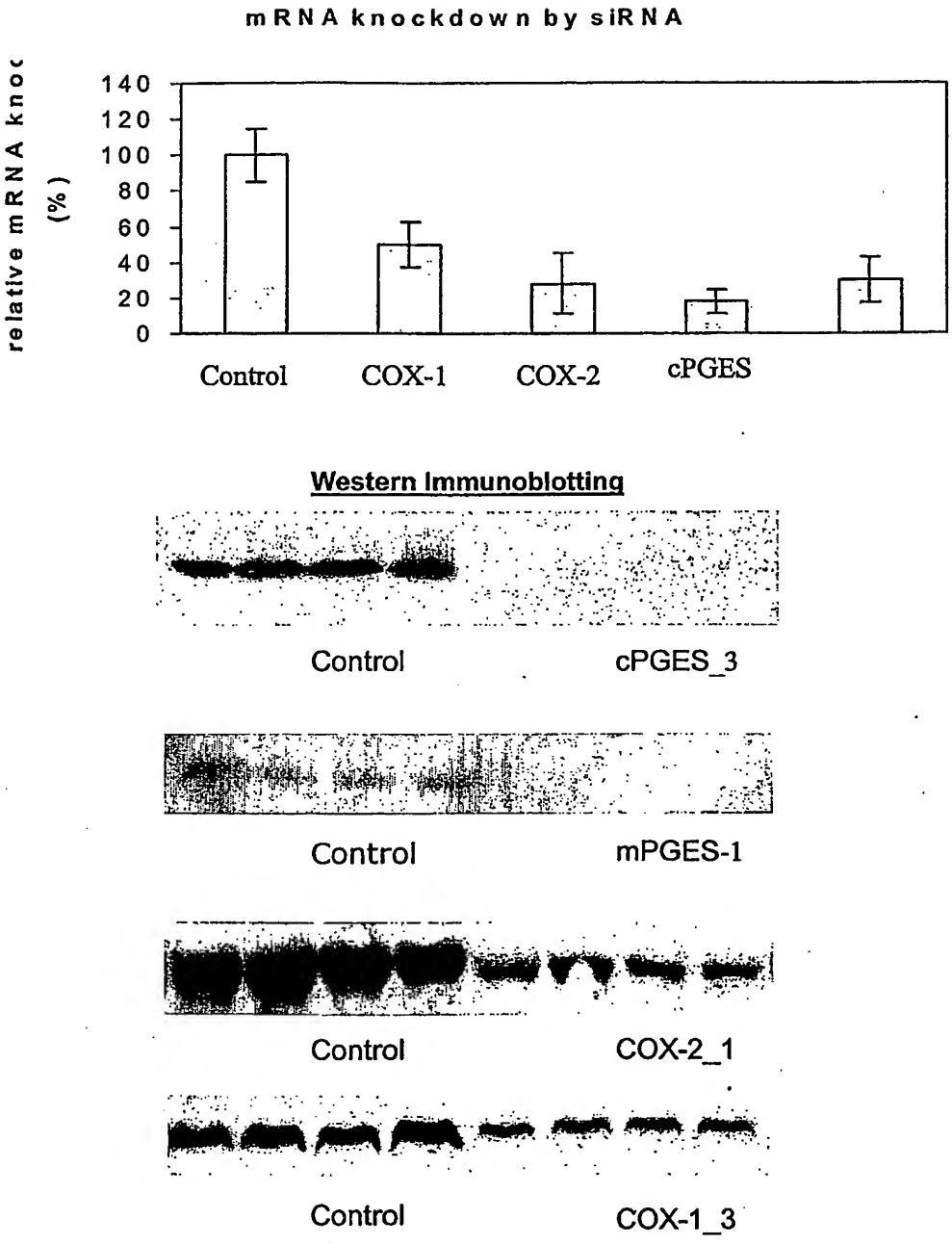
mRNA knockdown by siRNA
relative mRNA knoc
(%)
140
120
100
80
60
40
20
0
Control
COX-1
COX-2
cPGES
Western Immunoblotting
Control
cPGES_3
Control
mPGES-1
Control
COX-2_1
Control
COX-1_3

FIG. 8

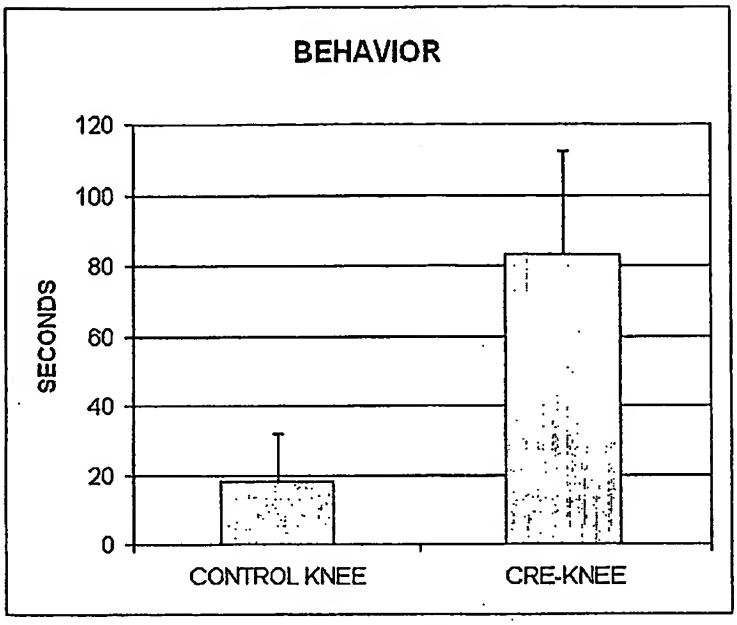
BEHAVIOR
SECONDS
120
100
80
60
40
20
0
CONTROL KNEE
CRE-KNEE

FIG. 9

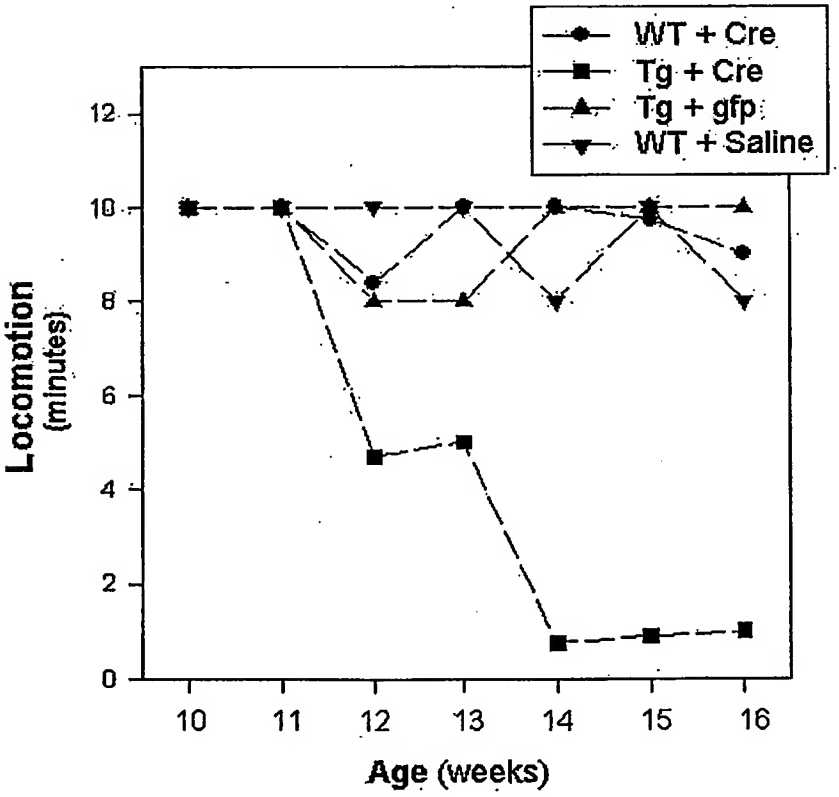
WT + Cre
Tg + Cre
Tg + gfp
WT + Saline
Locomotion
(minutes)
12
10
8
6
4
2
0
10
11
12
13
14
15
16
Age (weeks)

FIG. 10

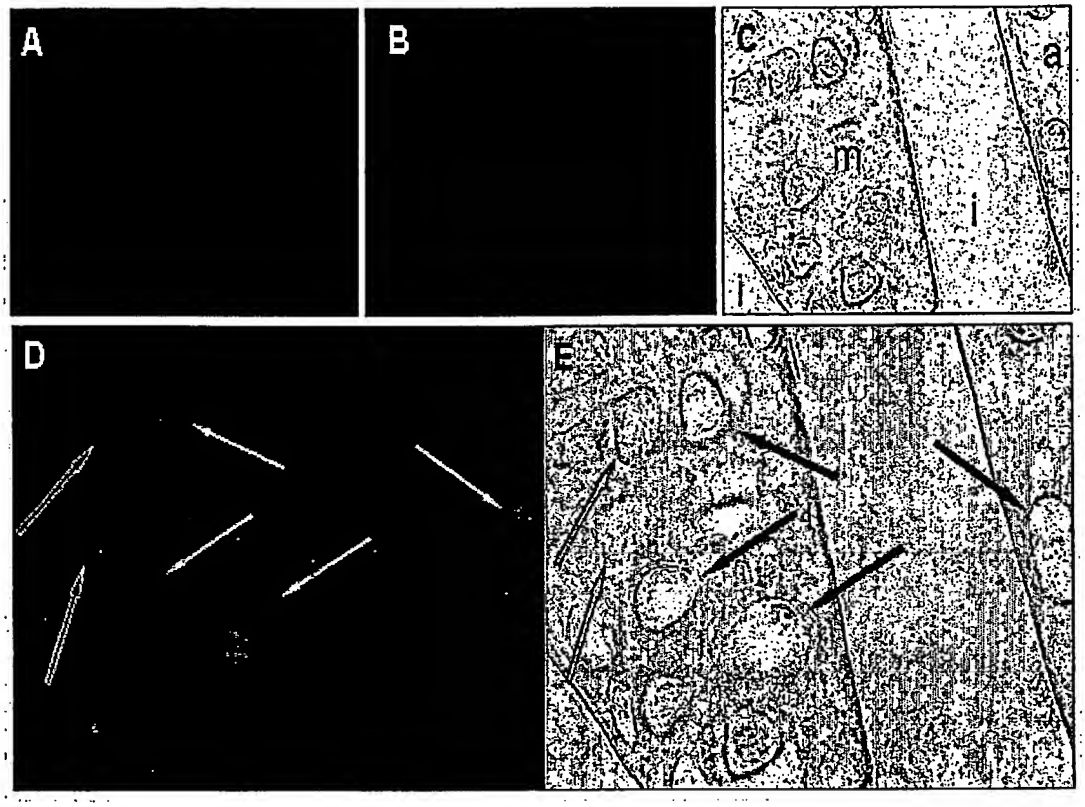

FIG. 11

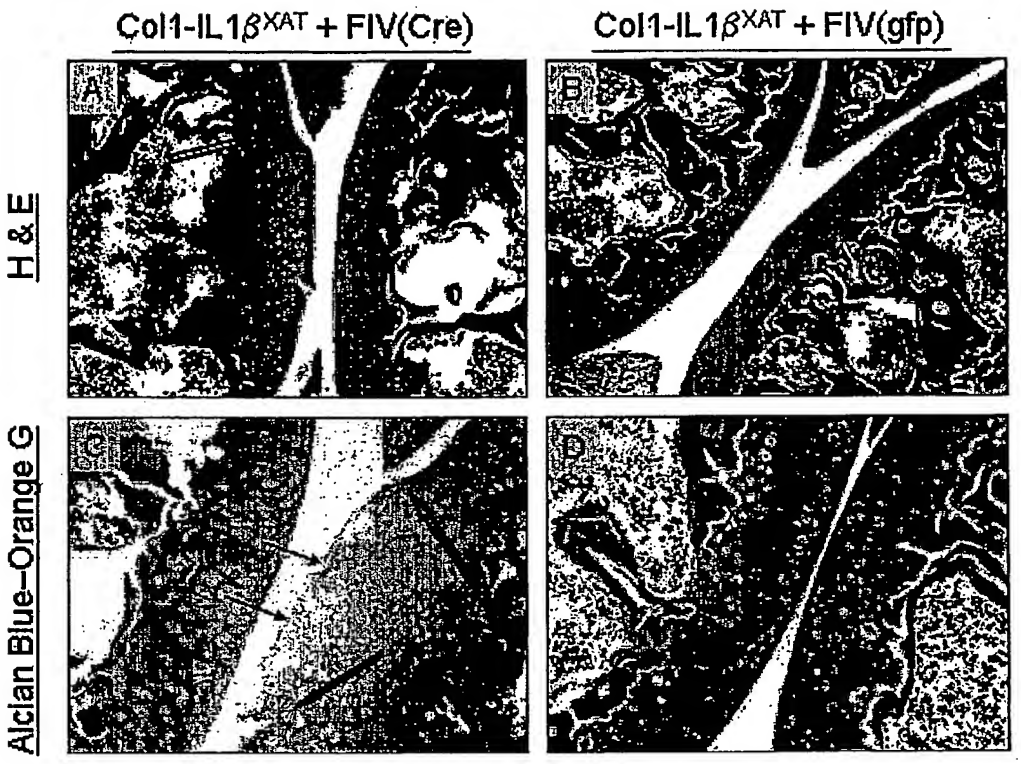
Col1-IL1β^XAT + FIV(Cre)
Col1-IL1β^XAT + FIV(gfp)
H&E
Alcian Blue–Orange G
A
B
C
D

FIG. 12

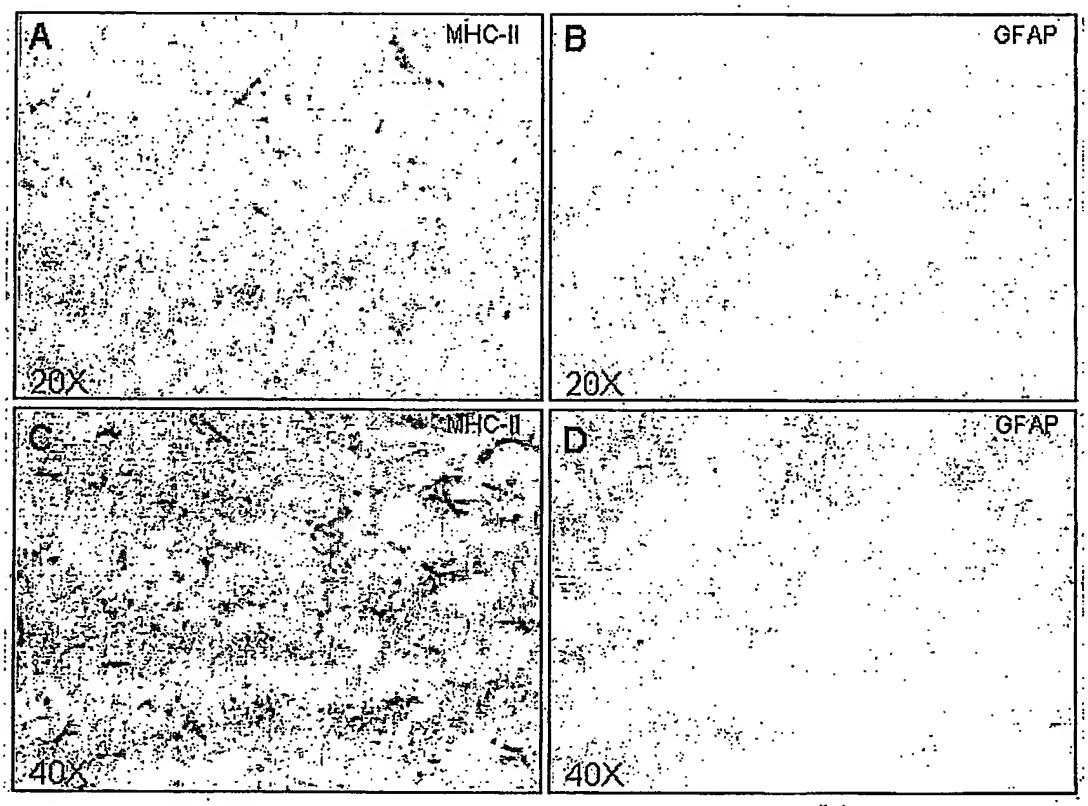

FIG. 13

FIG. 14

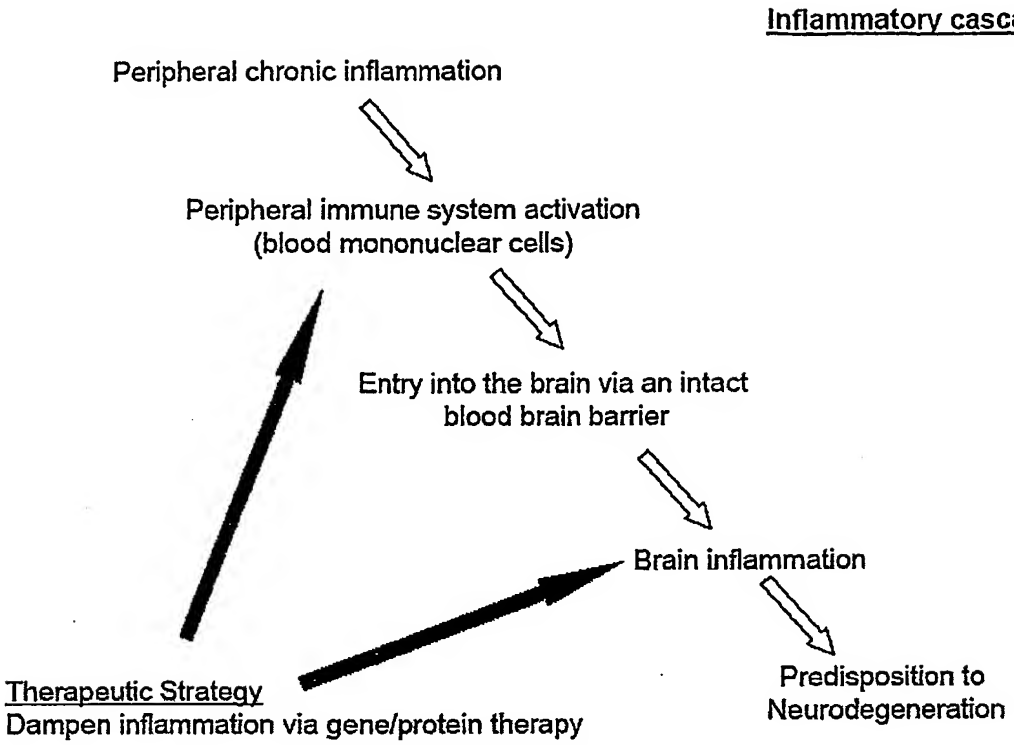
Inflammatory cascade
Peripheral chronic inflammation
Peripheral immune system activation
(blood mononuclear cells)
Entry into the brain via an intact
blood brain barrier
Brain inflammation
Predisposition to
Neurodegeneration
Therapeutic Strategy
Dampen inflammation via gene/protein therapy

FIG. 15

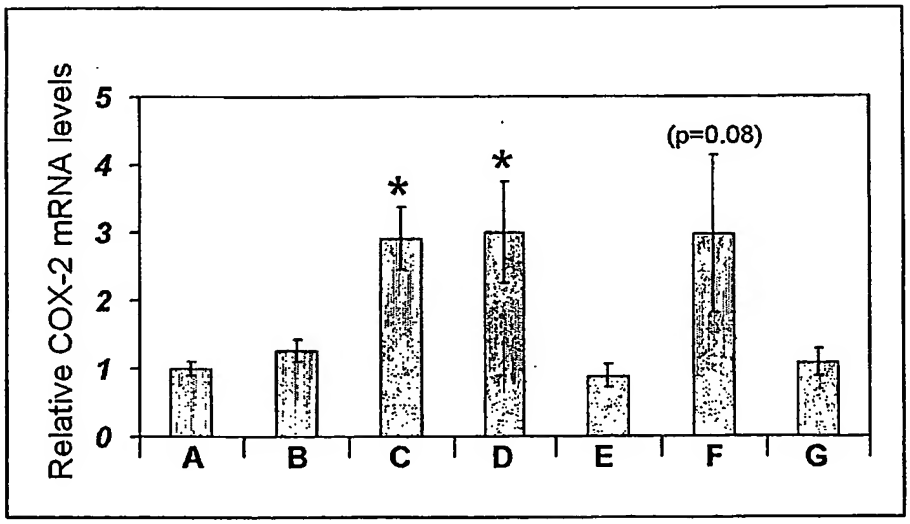
Relative COX-2 mRNA levels
0
1
2
3
4
5
*
*
(p=0.08)
A
B
C
D
E
F
G

FIG. 16

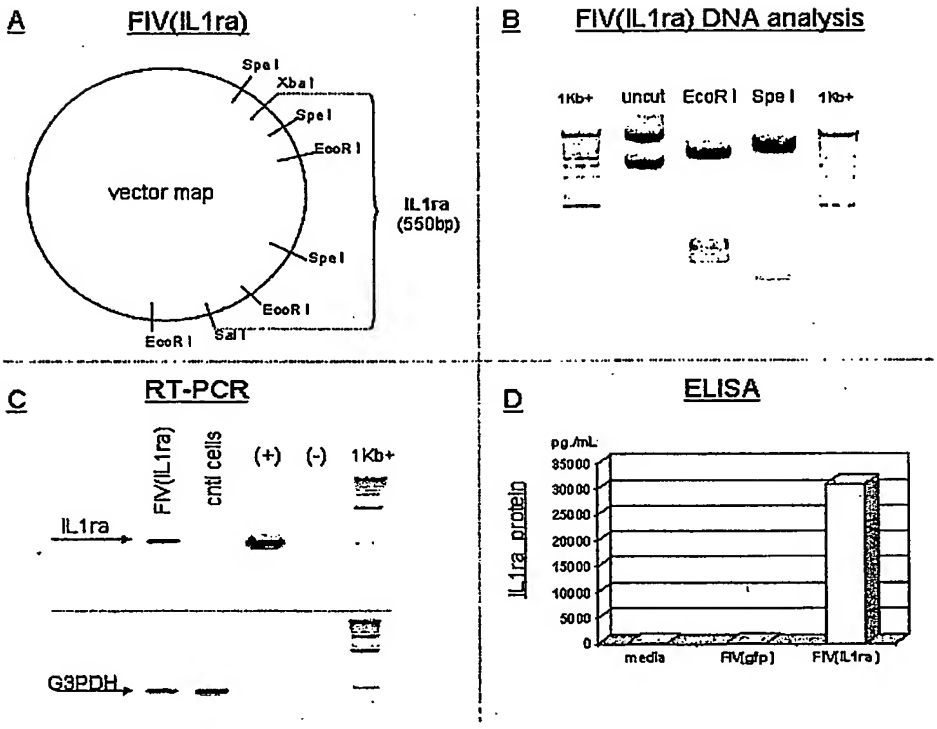
A
FIV(IL1ra)
SpeI
XbaI
SpeI
EcoR I
vector map
IL1ra
(550bp)
SpeI
EcoR I
EcoR I
SalI
B
FIV(IL1ra) DNA analysis
1Kb+
uncut
EcoR I
Spe I
1Kb+
C
RT-PCR
FIV(IL1ra)
cntl cells
(+)
(-)
1Kb+
IL1ra
G3PDH
D
ELISA
pg./mL
35000
30000
25000
20000
15000
10000
5000
0
IL1ra protein
media
FIV(gfp)
FIV(IL1ra)

FIG. 17

SEQUENCE LISTING

<110> UNIVERSITY OF ROCHESTER, et al.
KYRKANIDES, Stephanos

<120> COMPOSITIONS AND METHODS FOR STUDYING AND TREATING INFLAMMATORY DISEASES AND DISORDERS

<130> 21108/0054P1

<140> NEW
<141> 2006-01-20

<150> 60/646,098
<151> 2005-01-20

<150> 60/646,099
<151> 2005-01-20

<160> 82

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 2943
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note = synthetic construct

<400> 1
accaggcaac accattgaag gctcatatgt aaaaatccat gccttccttt ctcccaatct 60
ccattcccaa acttagccac tggcttctgg ctgaggcctt acgcatacct cccggggctt 120
gcacacacct tcttctacag aagacacacc ttgggcatat cctacagaag accaggcttc 180
tctctggtcc ttggtagagg gctactttac tgtaacaggg ccagggtgga gagttctctc 240
ctgaagctcc atcccctcta taggaaatgt gttgacaata ttcagaagag taagaggatc 300
aagacttctt tgtgctcaaa taccactgtt ctcttctcta ccctgcccta accaggagct 360
tgtcacccca aactctgagg tgatttatgc cttaatcaag caaacttccc tcttcagaaa 420
agatggctca ttttccctca aaagttgcca ggagctgcca agtattctgc caattcaccc 480
tggagcacaa tcaacaaatt cagccagaac acaactacag ctactattag aactattatt 540
attaataaat tcctctccaa atctagcccc ttgacttcgg atttcacgat ttctcccttc 600
ctcctagaaa cttgataagt ttcccgcgct tccctttttc taagactaca tgtttgtcat 660
cttataaagc aaaggggtga ataaatgaac caaatcaata acttctggaa tatctgcaaa 720
caacaataat atcagctatg ccatctttca ctattttagc cagtatcgag ttgaatgaac 780
atagaaaaat acaaaactga attcttccct gtaaattccc cgttttgacg acgcacttgt 840
agccacgtag ccacgcctac ttaagacaat tacaaaaggc gaagaagact gactcaggct 900
taagctgcca gccagagagg gagtcatttc attggcgttt gagtcagcaa agaagtcaag 960
atggccaaag ttccagacat gtttgaagac ctgaagaact gttacagtga aaatgaagaa 1020
gacagttcct ccattgatca tctgtctctg aatcagaaat ccttctatca tgtaagctat 1080
ggcccactcc atgaaggctg catggatcaa tctgtgtctc tgagtatctc tgaaacctct 1140
aaaacatcca agcttacctt caaggagagc atggtggtag tagcaaccaa cgggaaggtt 1200
ctgaagaaga gacggttgag tttaagccaa tccatcactg atgatgacct ggaggccatc 1260
gccaatgact cagaggaaga aatcatcaag cctaggtcag caccttttag cttcctgagc 1320
aatgtgaaat acaactttat gaggatcatc aaatacgaat tcatcctgaa tgacgccctc 1380
aatcaaagta taattcgagc caatgatcag tacctcacgg ctgctgcatt acataatctg 1440
gatgaagcag tgaaatttga catgggtgct tataagtcat caaaggatga tgctaaaatt 1500

accgtgattc taagaatctc aaaaactcaa ttgtatgtga ctgcccaaga tgaagaccaa 1560
ccagtgctgc tgaaggagat gcctgagata cccaaaacca tcacaggtag tgagaccaac 1620
ctcctcttct tctgggaaac tcacggcact aagaactatt tcacatcagt tgcccatcca 1680
aacttgttta ttgccacaaa gcaagactac tgggtgtgct tggcaggggg gccaccctct 1740
atcactgact ttcagatact ggaaaaccag gcgtaggtct ggagtctcac ttgtctcact 1800
tgtgcagtgt tgacagttca tatgtaccat gtacatgaag aagctaaatc ctttactgtt 1860
agtcatttgc tgagcatgta ctgagccttg taattctaaa tgaatgttta cactctttgt 1920
aagagtggaa ccaacactaa catataatgt tgttatttaa agaacaccct atattttgca 1980
tagtaccaat cattttaatt attattcttc ataacaattt taggaggacc agagctactg 2040
actatggcta ccaaaaagac tctacccata ttacagatgg gcaaattaag gcataagaaa 2100
actaagaaat atgcacaata gcagttgaaa caagaagcca cagacctagg atttcatgat 2160
ttcatttcaa ctgtttgcct tctacttta agttgctgat gaactcttaa tcaaatagca 2220
taagtttctg ggacctcagt tttatcattt tcaaaatgga gggaataata cctaagcctt 2280
cctgccgcaa cagtttttta tgctaatcag ggaggtcatt ttggtaaaat acttcttgaa 2340
gccgagcctc aagatgaagg caaagcacga aatgttattt tttaattatt atttatatat 2400
gtatttataa atatatttaa gataattata atatactata tttatgggaa ccccttcatc 2460
ctctgagtgt gaccaggcat cctccacaat agcagacagt gttttctggg ataagtaagt 2520
ttgatttcat taatacaggg cattttggtc caagttgtgc ttatcccata gccaggaaac 2580
tctgcattct agtacttggg agacctgtaa tcatataata aatgtacatt aattaccttg 2640
agccagtaat tggtccgatc tttgactctt ttgccattaa acttacctgg gcattcttgt 2700
ttcaattcca cctgcaatca agtcctacaa gctaaaatta gatgaactca actttgacaa 2760
ccatgagacc actgttatca aaactttctt ttctggaatg taatcaatgt ttcttctagg 2820
ttctaaaaat tgtgatcaga ccataatgtt acattattat caacaatagt gattgataga 2880
gtgttatcag tcataactaa ataaagcttg caacaaaatt ctctgacaaa aaaaaaaaaa 2940
aaa 2943

<210> 2
<211> 1498
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 2
accaaacctc ttcgaggcac aaggcacaac aggctgctct gggattctct tcagccaatc 60
ttcattgctc aagtgtctga agcagccatg gcagaagtac ctgagctcgc cagtgaaatg 120
atggcttatt acagtggcaa tgaggatgac ttgttctttg aagctgatgg ccctaaacag 180
atgaagtgct ccttccagga cctggacctc tgccctctgg atggcggcat ccagctacga 240
atctccgacc accactacag caagggcttc aggcaggccg cgtcagttgt tgtggccatg 300
gacaagctga ggaagatgct ggttccctgc ccacagacct tccaggagaa tgacctgagc 360
accttctttc ccttcatctt tgaagaagaa cctatcttct tcgacacatg ggataacgag 420
gcttatgtgc acgatgcacc tgtacgatca ctgaactgca cgctccggga ctcacagcaa 480
aaaagcttgg tgatgtctgg tccatatgaa ctgaaagctc tccacctcca gggacaggat 540
atggagcaac aagtggtgtt ctccatgtcc tttgtacaag gagaagaaag taatgacaaa 600
atacctgtgg ccttgggcct caaggaaaag aatctgtacc tgtcctgcgt gttgaaagat 660
gataagccca ctctacagct ggagagtgta gatcccaaaa attacccaaa gaagaagatg 720
gaaaagcgat ttgtcttcaa caagatagaa atcaataaca agctggaatt tgagtctgcc 780
cagttcccca actggtacat cagcacctct caagcagaaa acatgcccgt cttcctggga 840
gggaccaaag gcggccagga tataactgac ttcaccatgc aatttgtgtc ttcctaaaga 900
gagctgtacc cagagagtcc tgtgctgaat gtggactcaa tccctagggc tggcagaaag 960
ggaacagaaa ggtttttgag tacggctata gcctggactt tcctgttgtc tacaccaatg 1020
cccaactgcc tgccttaggg tagtgctaag aggatctcct gtccatcagc caggacagtc 1080
agctctctcc tttcagggcc aatccccagc ccttttgttg agccaggcct ctctcacctc 1140
tcctactcac ttaaagcccg cctgacagaa accacggcca catttggttc taagaaaccc 1200
tctgtcattc gctcccacat tctgatgagc aaccgcttcc ctatttattt atttatttgt 1260
ttgtttgttt tattcattgg tctaatttat tcaaaggggg caagaagtag cagtgtctgt 1320
aaaagagcct agtttttaat agctatggaa tcaattcaat ttggactggt gtgctctctt 1380
taaatcaagt cctttaatta agactgaaaa tatataagct cagattattt aaatgggaat 1440
atttataaat gagcaaatat catactgttc aatggttctg aaataaactt cactgaag 1498

<210> 3
<211> 464
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 3
gcacctgtac gatcactgaa ctgcacgctc cgggactcac agcaaaaaag cttggtgatg 60
tctggtccat atgaactgaa agctctccac ctccagggac aggatatgga gcaacaagtg 120
gtgttctcca tgtcctttgt acaaggagaa gaaagtaatg acaaaatacc tgtggccttg 180
ggcctcaagg aaaagaatct gtacctgtcc tgcgtgttga aagatgataa gcccactcta 240
cagctggaga gtgtagatcc caaaaattac ccaaagaaga agatggaaaa gcgatttgtc 300
ttcaacaaga tagaaatcaa taacaagctg gaatttgagt ctgcccagtt ccccaactgg 360
tacatcagca cctctcaagc agaaaacatg cccgtcttcc tgggagggac caaaggcggc 420
caggatataa ctgacttcac catgcaattt gtgtcttcct aaag 464

<210> 4
<211> 539
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 4
atggaaatct gcagaggcct ccgcagtcac ctaatcactc tcctcctctt cctgttccat 60
tcagagacga tctgcgcacc tgtacgatca ctgaactgca cgctccggga ctcacagcaa 120
aaaagcttgg tgatgtctgg tccatatgaa ctgaaagctc tccacctcca gggacaggat 180
atggagcaac aagtggtgtt ctccatgtcc tttgtacaag gagaagaaag taatgacaaa 240
atacctgtgg ccttgggcct caaggaaaag aatctgtacc tgtcctgcgt gttgaaagat 300
gataagccca ctctacagct ggagagtgta gatcccaaaa attacccaaa gaagaagatg 360
gaaaagcgat ttgtcttcaa caagatagaa atcaataaca agctggaatt tgagtctgcc 420
cagttcccca actggtacat cagcacctct caagcagaaa acatgcccgt cttcctggga 480
gggaccaaag gcggccagga tataactgac ttcaccatgc aatttgtgtc ttcctaaag 539

<210> 5
<211> 1760
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 5
atttctttat aaaccacaac tctgggcccg caatggcagt ccactgcctt gctgcagtca 60
cagaatggaa atctgcagag gcctccgcag tcacctaatc actctcctcc tcttcctgtt 120
ccattcagag acgatctgcc gaccctctgg gagaaaatcc agcaagatgc aagccttcag 180
aatctgggat gttaaccaga agaccttcta tctgaggaac aaccaactag ttgctggata 240
cttgcaagga ccaaatgtca atttagaaga aaagatagat gtggtaccca ttgagcctca 300
tgctctgttc ttgggaatcc atggagggaa gatgtgcctg tcctgtgtca agtctggtga 360
tgagaccaga ctccagctgg aggcagttaa catcactgac ctgagcgaga acagaaagca 420
ggacaagcgc ttcgccttca tccgctcaga cagcggcccc accaccagtt ttgagtctgc 480
cgcctgcccc ggttggttcc tctgcacagc gatggaagct gaccagcccg tcagcctcac 540
caatatgcct gacgaaggcg tcatggtcac caaattctac ttccaggagg acgagtagta 600
ctgcccaggc ctgcctgttc ccattcttgc atggcaagga ctgcagggac tgccagtccc 660

cctgccccag ggctcccggc tatgggggca ctgaggacca gccattgagg ggtggaccct 720
cagaaggcgt cacaagaacc tggtcacagg actctgcctc ctcttcaact gaccagcctc 780
catgctgcct ccagaatggt ctttctaatg tgtgaatcag agcacagcag cccctgcaca 840
aagcccttcc atgtcgcctc tgcattcagg atcaaacccc gaccacctgc ccaacctgct 900
ctcctcttgc cactgcctct tcctccctca ttccaccttc ccatgccctg gatccatcag 960
gccacttgat gacccccaac caagtggctc ccacaccctg ttttacaaaa aagaaaagac 1020
cagtccatga gggaggtttt taagggtttg tggaaaatga aaattaggat ttcatgattt 1080
tttttttca gtccccgtga aggagagccc ttcatttgga gattatgttc tttcggggag 1140
aggctgagga cttaaaatat tcctgcattt gtgaaatgat ggtgaaagta agtggtagct 1200
tttcccttct ttttcttctt tttttgtgat gtcccaactt gtaaaaatta aaagttatgg 1260
tactatgtta gccccataat tttttttttc cttttaaaac acttccataa tctggactcc 1320
tctgtccagg cactgctgcc cagcctccaa gctccatctc cactccagat tttttacagc 1380
tgcctgcagt actttacctc ctatcagaag tttctcagct cccaaggctc tgagcaaatg 1440
tggctcctgg gggttctttc ttcctctgct gaaggaataa attgctcctt gacattgtag 1500
agcttctggc acttggagac ttgtatgaaa gatggctgtg cctctgcctg tctcccccac 1560
cgggctggga gctctgcaga gcaggaaaca tgactcgtat atgtctcagg tccctgcagg 1620
gccaagcacc tagcctcgct cttggcaggt actcagcgaa tgaatgctgt atatgttggg 1680
tgcaaagttc cctacttcct gtgacttcag ctctgtttta caataaaatc ttgaaaatgc 1740
ctaaaaaaaa aaaaaaaaaa 1760

<210> 6
<211> 534
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 6
atggaaatct gcagaggcct ccgcagtcac ctaatcactc tcctcctctt cctgttccat 60
tcagagacga tctgccgacc ctctgggaga aaatccagca agatgcaagc cttcagaatc 120
tgggatgtta accagaagac cttctatctg aggaacaacc aactagttgc tggatacttg 180
caaggaccaa atgtcaattt agaagaaaag atagatgtgg tacccattga gcctcatgct 240
ctgttcttgg gaatccatgg agggaagatg tgcctgtcct gtgtcaagtc tggtgatgag 300
accagactcc agctggaggc agttaacatc actgacctga gcgagaacag aaagcaggac 360
aagcgcttcg ccttcatccg ctcagacagc ggccccacca ccagttttga gtctgccgcc 420
tgccccggtt ggttcctctg cacagcgatg gaagctgacc agcccgtcag cctcaccaat 480
atgcctgacg aaggcgtcat ggtcaccaaa ttctacttcc aggaggacga gtag 534

<210> 7
<211> 534
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 7
atggaaatct gcagaggcct ccgcagtcac ctaatcactc tcctcctctt cctgttccat 60
tcagagacga tctgccgacc ctctgggaga aaatccagca agatgcaagc cttcagaatc 120
tgggatgtta accagaagac cttctatctg aggaacaacc aactagttgc tggatacttg 180
caaggaccaa atgtcaattt agaagaaaag atagatgtgg tacccattga gcctcatgct 240
ctgttcttgg gaatccatgg agggaagatg tgcctgtcct gtgtcaagtc tggtgatgag 300
accagactcc agctggaggc agttaacatc actgacctga gcgagaacag aaagcaggac 360
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tgccccggtt ggttcctctg cacagcgatg gaagctgacc agcccgtcag cctcaccaat 480
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<210> 8

<211> 4849
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 8
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gtcatctgca aatgaaattg atgttcgtcc ctgtcctctt aacccaaatg aacacaaagg 240
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<210> 9
<211> 1436
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 9
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gccacgtgct gctgggtctc agtcctccac ttcccgtgtc ctctggaagt tgtcaggagc 180
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<210> 10
<211> 2554
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 10
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catgccagca ccagggcatc tgtgtccgct tcggccttga ccgctaccag tgtgactgca 180
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<210> 11
<211> 4465
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

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tgcccgccgc tcggatgctc gcccgcgccc tgctgctgtg cgcggtcctg gcgctcagcc 180
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gatttgacca gtataagtgc gattgtaccc ggacaggatt ctatggagaa aactgctcaa 300
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agaaatagtc aatatgcttg tataaaacac tgttcactgt ttttttaaa aaaaaaactt 4320
gatttgttat taacattgat ctgctgacaa aacctgggaa tttgggttgt gtatgcgaat 4380
gtttcagtgc ctcagacaaa tgtgtattta acttatgtaa aagataagtc tggaaataaa 4440
tgtctgttta tttttgtact attta 4465

<210> 12
<211> 1805
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 12
gctgctcctc tgtcgagctg atcacaccca cagttgagct gcgctggcca gagatgcctg 60
cccacagcct ggtgatgagc agcccggccc tcccggcctt cctgctctgc agcacgctgc 120
tggtcatcaa gatgtacgtg gtggccatca tcacgggcca agtgaggctg cggaagaagg 180
cctttgccaa ccccgaggat gccctgagac acggaggccc ccagtattgc aggagcgacc 240
ccgacgtgga acgctgcctc agggcccacc ggaacgacat ggagaccatc tacccccttcc 300
ttttcctggg cttcgtctac tcctttctgg gtcctaaccc ttttgtcgcc tggatgcact 360
tcctggtctt cctcgtgggc cgtgtggcac acaccgtggc ctacctgggg aagctgcggg 420
cacccatccg ctccgtgacc tacaccctgg cccagctccc ctgcgcctcc atggctctgc 480
agatcctctg ggaagcggcc cgccacctgt gaccagcagc tgatgcctcc ttggccacca 540
gaccatgggc caagagccgc cgtggctata cctggggact tgatgttcct tccagattgt 600
ggtgggccct gagtcctggt ttcctggcag cctgctgcgc gtgtgggtct ctgggcacag 660
tgggcctgtg tgtgtgcccg tgtgtgtgta tgtgtgtgtg tatgtttctt agccccttgg 720
attcctgcac gaagtggctg atgggaacca tttcaagaca gattgtgaag attgatagaa 780
aatccttcag ctaaagtaac agagcatcaa aaacatcact ccctctccct ccctaacagt 840
gaaaagagag aagggagact ctatttaaga ttcccaaacc taatgatcat ctgaatcccg 900
ggctaagaat gcagactttt cagactgacc ccagaaattc tggcccagcc aatctagagg 960
caagcctggc catctgtatt ttttttttc caagacagag tcttgctctg ttgcccaagc 1020
tggagtgaag tggtacaatc tggctcactg cagcctccgc ctcccgggtt caagcgattc 1080
tcccgcctca gcctcctgag tagctgggat tacaggcgcg tatcaccata cccagctaat 1140
ttttgtattt ttagtagaga cgggttcacc atgttgccca ggagggtctc gaactcctgg 1200
cctcaagtga tccaccggcc tcggcctccc aaagtgctgg gatgacaggc atgaatcact 1260
gtgctcagcc accatctgga gttttaaaag gctcccatgt gagtccctgt gatggccagg 1320
ccaggggacc cctgccagtt ctctgtggaa gcaaggctgg ggtcttgggt tcctgtatgg 1380
tggaagctgg gtgagccaag gacagggctg gctcctctgc ccccgctgac gcttcccttg 1440
ccgttggctt tggatgtctt tgctgcagtc ttctctctgg ctcaggtgtg ggtgggaggg 1500
gcccacagga agctcagcct tctcctccca aggtttgagt ccctccaaag ggcagtgggt 1560

ggaggaccgg gagctttggg tgaccagcca ctcaaaggaa ctttctggtc ccttcagtat 1620
cttcaaggtt tggaaactgc aaatgtcccc ttgatgggga atccgtgtgt gtgtgtgtgt 1680
gtgtgtgtgt gtgtgtgtgt gtgtgtgttt tctcctagac ccgtgacctg agatgtgtga 1740
tttttagtca ttaaatggaa gtgtctgcca gctgggccca gcacctaaaa aaaaaaaaaa 1800
aaaaa 1805

<210> 13
<211> 782
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequences; note =
synthetic construct

<400> 13
ggattcgggc tacactttcc tcttctcccc gaccggagag ccgctctttc cgcgcggtgc 60
attctggggc ccgaggtcga gcccgccgct gccgccgtcg cctgagggaa gcgagaagag 120
gccgcgaccg agagaaaaag cggagtcgca ccggagagaa gtcgactccc tagcagcagc 180
cgccgccaga gagcccgccc accagttcgc ccgtcccccc gccccgttca caatgcagcc 240
tgcttctgca aagtggtacg atcgaaggga ctatgtcttc attgaatttt gtgttgaaga 300
cagtaaggat gttaatgtaa attttgaaaa atccaaactt acattcagtt gtctcggagg 360
aagtgataat tttaagcatt taaatgaaat tgatcttttt cactgtattg atccaaatga 420
ttccaagcat aaaagaacgg acagatcaat tttatgttgt ttacgaaaag gagaatctgg 480
ccagtcatgg ccaaggttaa caaaagaaag ggcaaagctt aattggctta gtgtcgactt 540
caataattgg aaagactggg aagatgattc agatgaagac atgtctaatt ttgatcgttt 600
ctctgagatg atgaacaaca tgggtggtga tgaggatgta gatttaccag aagtagatgg 660
agcagatgat gattcacaag acagtgatga tgaaaaaatg ccagatctgg agtaaggaat 720
attgtcatca cctggatttt gagaaagaaa aataacttct ctgcaagatt tcataattga 780
ga 782

<210> 14
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 14
atggaaatct gcagaggcct ccgcagtcac ctaatcactc tcctcctctt cctgttccat 60
tcagagacga tctgc 75

<210> 15
<211> 655
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 15
cgatgtacgg gccagatata cgcgttgaca ttgattattg actagttatt aatagtaatc 60
aattacgggg tcattagttc atagcccata tatggagttc cgcgttacat aacttacggt 120
aaatggcccg cctggctgac cgcccaacga cccccgccca ttgacgtcaa taatgacgta 180
tgttcccata gtaacgccaa tagggacttt ccattgacgt caatgggtgg actatttacg 240
gtaaactgcc cacttggcag tacatcaagt gtatcatatg ccaagtacgc cccctattga 300
cgtcaatgac ggtaaatggc ccgcctggca ttatgcccag tacatgacct tatgggactt 360

tcctacttgg cagtacatct acgtattagt catcgctatt accatggtga tgcggttttg 420
gcagtacatc aatgggcgtg gatagcggtt tgactcacgg ggatttccaa gtctccaccc 480
cattgacgtc aatgggagtt tgttttggca ccaaaatcaa cgggactttc caaaatgtcg 540
taacaactcc gccccattga cgcaaatggg cggtaggcgt gtacggtggg aggtctatat 600
aagcagagct ctctggctaa ctagagaacc cactgcttac tggcttatcg aaatt 655

<210> 16
<211> 1278
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 16
tcgaggtgag ccccacgttc tgcttcactc tccccatctc ccccccctcc ccacccccaa 60
ttttgtattt atttatttt taattatttt gtgcagcgat gggggcgggg gggggggggg 120
cgcgcgccag gcggggcggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg 180
gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg 240
cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag tcgctgcgtt gccttcgccc 300
cgtgccccgc tccgcgccgc ctcgcgccgc ccgccccggc tctgactgac cgcgttactc 360
ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg cttggtttaa 420
tgacggctcg tttcttttct gtggctgcgt gaaagcctta aagggctccg ggagggccct 480
ttgtgcgggg gggagcggct cgggggggtgc gtgcgtgtgt gtgtgcgtgg ggagcgccgc 540
gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg gctttgtgcg 600
ctccgcgtgt gcgcgagggg agcgcggccg ggggcggtgc cccgcggtgc gggggggctg 660
cgaggggaac aaaggctgcg tgcggggtgt gtgcgtgggg gggtgagcag ggggtgtggg 720
cgcggcggtc gggctgtaac ccccccctgc acccccctcc ccgagttgct gcgcacggcc 780
cggcttcggg tgcggggctc cgtgcggggc gtggcgcggg gctcgccgtg ccgggcgggg 840
ggtggcggca ggtgggggtg ccgggcgggg cggggccgcc tcgggccggg gagggctcgg 900
gggaggggcg cggcggcccc ggagcgccgg cggctgtcga ggcgcggcga gccgcagcca 960
ttgcctttta tggtaatcgt gcgagagggc gcagggactt cctttgtccc aaatctggcg 1020
gagccgaaat ctgggaggcg ccgccgcacc ccctctagcg ggcgcgggcg aagcggtgcg 1080
gcgccggcag gaaggaaatg ggcggggagg gccttcgtgc gtcgccgcgc cgccgtcccc 1140
ttctccatct ccagcctcgg ggctgccgca gggggacggc tgccttcggg ggggacgggg 1200
cagggcgggg ttcggcttct ggcgttgtac cggcggggtt tatatcttcc cttctctgtt 1260
cctccgcagc cagccatg 1278

<210> 17
<211> 1176
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 17
cccgggccca gcaccccaag gcggccaacg ccaaaactct ccctcctcct cttcctcaat 60
ctcgctctcg ctctttttt ttttcgcaaa aggaggggag agggggtaaa aaaatgctgc 120
actgtgcggc gaagccggtg agtgagcggc gcggggccaa tcagcgtgcg ccgttccgaa 180
agttgccttt tatggctcga gcggccgcgg cggcgcccta taaaacccag cggcgcgacg 240
cgccaccacc gccgagaccg cgtccgcccc gcgagcacag agcctcgcct ttgccgatcc 300
gccgcccgtc cacacccgcc gccaggtaag cccggccagc cgaccggggc atgcggccgc 360
ggcccccttcg cccgtgcaga gccgccgtct gggccgcagc gggggggcgca tgggggggga 420
accggaccgc cgtgggggggc gcgggagaag cccctgggcc tccggagatg ggggacaccc 480
cacgccagtt cggaggcgcg aggccgcgct cgggaggcgc gctccgggggg tgccgctctc 540
ggggcggggg caaccggcgg ggtctttgtc tgagccgggc tcttgccaat ggggatcgca 600
gggtgggcgc ggcgtagccc ccgccaggcc cggtgggggc tggggcgcca ttgccggtgc 660
gcgctggtcc tttgggcgct aactgcgtgc gcgctgggaa ttggcgctaa ttgcgcgtgc 720

gcgctgggac tcaaggcgct aattgcgcgt gcgttctggg gcccggggtg ccgcggcctg 780
ggctggggcg aaggcgggct cggccggaag gggtggggtc gccgcggctc ccgggcgctt 840
gcgcgcactt cctgcccgag ccgctggccg cccgagggtg tggccgctgc gtgcgcgcgc 900
gccgacccgg cgctgtttga accgggcgga ggcggggctg gcgcccggtt gggaggggt 960
tggggcctgg cttcctgccg cgcgccgcgg ggacgcctcc gaccagtgtt tgcctttttat 1020
ggtaataacg cggccggccc ggcttccttt gtccccaatc tgggcgcgcg ccggcgcccc 1080
ctggcggcct aaggactcgg cgcgccggaa gtggccaggg cgggggcgac ttcggctcac 1140
agcgcgcccg gctattctcg cagctcacca tggatg 1176

<210> 18
<211> 1729
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 18
gaattcggta ccctagttat taatagtaat caattacggg gtcattagtt catagcccat 60
atatggagtt ccgcgttaca taacttacgg taaatggccc gcctggctga ccgcccaacg 120
acccccgccc attgacgtca ataatgacgt atgttcccat agtaacgcca atagggactt 180
tccattgacg tcaatgggtg gactatttac ggtaaactgc ccacttggca gtacatcaag 240
tgtatcatat gccaagtacg ccccctattg acgtcaatga cggtaaatgg cccgcctggc 300
attatgccca gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag 360
tcatcgctat taccatggtc gaggtgagcc ccacgttctg cttcactctc cccatctccc 420
cccctcccc acccccaatt ttgtatttat ttatttttta attattttgt gcagcgatgg 480
gggcgggggg gggggggggg cgcgcgccag gcggggcggg gcggggcgag gggcggggcg 540
gggcgaggcg gagaggtgcg gcggcagcca atcagagcgg cgcgctccga aagtttcctt 600
ttatggcgag gcggcggcgg cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag 660
tcgctgcgac gctgccttcg ccccgtgccc cgctccgccg ccgcctcgcg ccgcccgccc 720
cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg 780
ggctgtaatt agcgcttggt ttaatgacgg cttgtttctt ttctgtggct gcgtgaaagc 840
cttgaggggc tccgggaggg ccctttgtgc ggggggggagc ggctcggggg gtgcgtgcgt 900
gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc 960
gggcgcggcg cggggctttg tgcgctccgc agtgtgcgcg aggggagcgc ggccgggggc 1020
ggtgccccgc ggtgcggggg gggctgcgag gggaacaaag gctgcgtgcg gggtgtgtgc 1080
gtgggggggt gagcaggggg tgtgggcgcg gcggtcgggc tgtaaccccc ccctgcaccc 1140
ccctccccga gttgctgagc acggcccggc ttcgggtgcg gggctccgta cggggcgtgg 1200
cgcggggctc gccgtgccgg gcgggggggtg gcggcaggtg ggggtgccgg gcggggcggg 1260
gccgcctcgg gccggggagg gctcggggga ggggcgcggc ggcccccgga gcgccggcgg 1320
ctgtcgaggc gcggcgagcc gcagccattg ccttttatgg taatcgtgcg agagggcgca 1380
gggacttcct ttgtcccaaa tctgtgcgga gccgaaatct gggaggcgcc gccgcacccc 1440
ctctagcggg cgcggggcga agcggtgcgg cgccggcagg aaggaaatgg gcggggaggg 1500
ccttcgtgcg tcgccgcgcc gccgtcccct tctccctctc cagcctcggg gctgtccgcg 1560
gggggacggc tgccttcggg ggggacgggg cagggcgggg ttcggcttct ggcgtgtgac 1620
cggcggctct agagcctctg ctaaccatgt tcatgccttc ttctttttcc tacagctcct 1680
gggcaacgtg ctggttattg tgctgtctca tcattttggc aaagaattc 1729

<210> 19
<211> 655
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 19
cgatgtacgg gccagatata cgcgttgaca ttgattattg actagttatt aatagtaatc 60
aattacgggg tcattagttc atagcccata tatggagttc cgcgttacat aacttacggt 120

aaatggcccg cctggctgac cgcccaacga cccccgccca ttgacgtcaa taatgacgta 180
tgttcccata gtaacgccaa tagggacttt ccattgacgt caatgggtgg actatttacg 240
gtaaactgcc cacttggcag tacatcaagt gtatcatatg ccaagtacgc cccctattga 300
cgtcaatgac ggtaaatggc ccgcctggca ttatgcccag tacatgacct tatgggactt 360
tcctacttgg cagtacatct acgtattagt catcgctatt accatggtga tgcggttttg 420
gcagtacatc aatgggcgtg gatagcggtt tgactcacgg ggatttccaa gtctccaccc 480
cattgacgtc aatgggagtt tgttttggca ccaaaatcaa cgggactttc caaaatgtcg 540
taacaactcc gccccattga cgcaaatggg cggtaggcgt gtacggtggg aggtctatat 600
aagcagagct ctctggctaa ctagagaacc cactgcttac tggcttatcg aaatt 655

<210> 20
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 20
tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg 60
cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt 120
gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 180
atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 240
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta 300
catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac 360
catggt 366

<210> 21
<211> 1295
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 21
ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc gggggggggg 60
gggggcgcg cgccaggcgg ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga 120
ggtgcggcgg cagccaatca gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg 180
cggcggcggc ggccctataa aaagcgaagc gcgcggcggg cgggagtcgc tgcgacgctg 240
ccttcgcccc gtgccccgct ccgccgccgc ctcgcgccgc ccgccccggc tctgactgac 300
cgcgttactc ccacaggtga gcgggcggga cggcccttct cctccgggct gtaattagcg 360
cttggtttaa tgacggcttg tttcttttct gtggctgcgt gaaagccttg aggggctccg 420
ggagggccct ttgtgcgggg gggagcggct cgggggtgc gtgcgtgtgt gtgtgcgtgg 480
ggagcgccgc gtgcggcccg cgctgcccgg cggctgtgag cgctgcgggc gcggcgcggg 540
gctttgtgcg ctccgcagtg tgcgcgaggg gagcgcggcc gggggcggtg ccccgcggtg 600
cgggggggc tgcgagggga acaaaggctg cgtgcggggt gtgtgcgtgg gggggtgagc 660
agggggtgtg ggcgcggcgg tcgggctgta acccccccct gcacccccct ccccgagttg 720
ctgagcacgg cccggcttcg ggtgcgggc tccgtacggg gcgtggcgcg gggctcgccg 780
tgccgggcgg ggggtggcgg caggtggggg tgccgggcgg ggcgggggccg cctcgggccg 840
gggagggctc gggggagggg cgcggcggcc cccggagcgc cggcggctgt cgaggcgcgg 900
cgagccgcag ccattgcctt ttatggtaat cgtgcgagag ggcgcaggga cttcctttgt 960
cccaaatctg tgcggagccg aaatctggga ggcgccgccg cacccccctct agcgggcgcg 1020
gggcgaagcg gtgcggcgcc ggcaggaagg aaatgggcgg ggagggcctt cgtgcgtcgc 1080
cgcgccgccg tcccccttctc cctctccagc ctcggggctg tccgcggggg gacggctgcc 1140
ttcgggggggg acggggcagg gcggggttcg gcttctggcg tgtgaccggc ggctctagag 1200
cctctgctaa ccatgttcat gccttcttct ttttcctaca gctcctgggc aacgtgctgg 1260
ttattgtgct gtctcatcat tttggcaaag aattc 1295

<210> 22
<211> 229
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 22
gtattagtca tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga 60
tagcggtttg actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg 120
ttttggcacc aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg 180
caaatgggcg gtaggcgtgt acggtgggag gtctatataa gcagagctc 229

<210> 23
<211> 281
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 23
tggcattatg cccagtacat gaccttatgg gactttccta cttggcagta catctacgta 60
ttagtcatcg ctattaccat ggtgatgcgg ttttggcagt acatcaatgg gcgtggatag 120
cggtttgact cacggggatt tccaagtctc caccccattg acgtcaatgg gagtttgttt 180
tggcaccaaa atcaacggga ctttccaaaa tgtcgtaaca actccgcccc attgacgcaa 240
atgggcggta ggcgtgtacg gtgggaggtc tatataagca g 281

<210> 24
<211> 282
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 24
attatgccca gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag 60
tcatcgctat taccatggtg atgcggtttt ggcagtacat caatgggcgt ggatagcggt 120
ttgactcacg gggatttcca agtctccacc ccattgacgt caatgggagt ttgttttggc 180
accaaaatca acgggacttt ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg 240
gcggtaggcg tgtacggtgg gaggtctata taagcagagc tc 282

<210> 25
<211> 512
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 25
ttgcgttaca taacttacgg taaatggccc gcctggctga ccgcccaacg acccccgccc 60
attgacgtca ataatgacgt atgttcccat agtaacgcca atagggactt tccattgacg 120
tcaatgggtg gactatttac ggtaaactgc ccacttggca gtacatcaag tgtatcatat 180
gccaagtacg ccccctattg acgtcaatga cggtaaatgg cccgcctggc attatgccca 240

gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag tcatcgctat 300
taccatggtg atgcggtttt ggcagtacat caatgggcgt ggatagcggt ttgactcacg 360
gggatttcca agtctccacc ccattgacgt caatgggagt ttgttttggc accaaaatca 420
acgggacttt ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg gcggtaggcg 480
tgtacggtgg gaggtctata taagcagagc tc 512

<210> 26
<211> 308
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 26
tcggcgaagc ctcgcgcggc cggccaggac gaggagcgcc actaggttga acatccgcac 60

gagccgccgg gccaggtctc ggacgggctc tcgagactcg atctcgtgca tgtcggcggt 120
ccgcggtgag gttatagacc atctgctagg cgggtccggg gagacaggca cattactggc 180
ctcggcgccc agcctaggcg tgtctagagc tcgaccgcgc gtccggagcg ccattcgacc 240
ggcgggtagc gagaagaacg ccggagaccg caggttataa caacgtcatg cataaattaa 300
gaatgggc 308

<210> 27
<211> 1848
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 27
ctgcagtgaa taataaaatg tgtgtttgtc cgaaatacgc gtttgagatt tctgtcccga 60
ctaaattcat gtcgcgcgat agtggtgttt atcgccgata gagatggcga tattggaaaa 120
atcgatattt gaaaatatgg catattgaaa atgtcgccga tgtgagtttc tgtgtaactg 180
atatcgccat ttttccaaaa gttgattttt gggcatacgc gatatctggc gatacgctta 240
tatcgtttac gggggatggc gatagacgcc tttggtgact tgggcgattc tgtgtgtcgc 300
aaatatcgca gtttcgatat aggtgacaga cgatatgagg ctatatcgcc gatagaggcg 360
acatcaagct ggcacatggc caatgcatat cgatctatac attgaatcaa tattggccat 420
tagccatatt attcattggt tatatagcat aaatcaatat tggctattgg ccattgcata 480
cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca ttaccgccat 540
gttgacattg attattgact agttattaat agtaatcaat tacggggtca ttagttcata 600
gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc 660
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<210> 28
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 28
cttctggcgt gtgaccggcg gggtttatat cttcccttcc caagcttgg 49

<210> 29
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 29
cttctggcgt gtgaccggcg gggtttatat cttcccttct ctgttcctcc gcagccccaa 60
gcttgg 66

<210> 30
<211> 68
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 30
cttctggcgt gtgaccggcg gggtttatat cttcccttct ctgttcctcc gcagccagcc 60
aagcttgg 68

<210> 31
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 31
cttctggcgt gtgaccggcg gggtttatat cttcccttct ctgttcctcc gcagccagcc 60
atggatgat 69

<210> 32
<211> 1345
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 32
tcgaggtgag ccccacgttc tgcttcactc tccccatctc ccccccctcc ccacccccaa 60
ttttgtattt atttattttt taattatttt gtgcagcgat gggggcgggg gggggggggg 120
cgcgcgccag gcggggcggg gcggggcgag gggcggggcg gggcgaggcg gagaggtgcg 180
gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag gcggcggcgg 240
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cagggcgggg ttcggcttct ggcgtgtgac cggcggctct agagcctctg ctaaccatgt 1260
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tcattttggc aaagaattca agctt 1345

<210> 33
<211> 684
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 33
tcaatattgg ccattagcca tattattcat tggttatata gcataaatca atattggcta 60
ttggccattg catacgttgt atctatatca taatatgtac atttatattg gctcatgtcc 120
aatatgaccg ccatgttggc attgattatt gactagttat taatagtaat caattacggg 180
gtcattagtt catagcccat atatggagtt ccgcgttaca taacttacgg taaatggccc 240
gcctggctga ccgcccaacg acccccgccc attgacgtca ataatgacgt atgttcccat 300
agtaacgcca atagggactt tccattgacg tcaatgggtg gagtatttac ggtaaactgc 360
ccacttggca gtacatcaag tgtatcatat gccaagtccg ccccctattg acgtcaatga 420
cggtaaatgg cccgcctggc attatgccca gtacatgacc ttacgggact ttcctacttg 480
gcagtacatc tacgtattag tcatcgctat taccatggtg atgcggtttt ggcagtacac 540
caatgggcgt ggatagcggt ttgactcacg gggatttcca agtctccacc ccattgacgt 600
caatgggagt ttgttttggc accaaaatca acgggacttt ccaaaatgtc gtaataaccc 660
cgccccgttg acgcaaatgg gcgg 684

<210> 34
<211> 2069
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 34
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aaggtcactg gcaacctctc tcaagatctc cctcactatg ccattattca ggattgggga 120
agatgtggct ggagcctaag gggctcttcc cttccctatg gtgggactca ttaggagaac 180
ctcagcaagc agtccactgt aagctcaaac aaataccatg tcgctggtat ggagtaaggc 240
tgttgctatg acaggaactc aggggtctta actggcttga gcgctgggag ggggcagcag 300
ccaggccttg tctgtaagct gaagacctgg cagtgctgag ctggtcaccc cccaggacct 360
ccttttgtgc ccaacgagtg actcaccttg ggcatagaca taatggtcag gggtgggcac 420
gcagcctgct tcccgctgtg ctccaggcct ccttcgatgc tttccgagaa gtctattgag 480
ctgggagctt gtactgcacc cggggctgac atcctggcat cctgggataa aagcagccca 540
cggggctgcc cttgccatat gcctcactgg cggcagagaa caaggctcta ttcagcaagt 600
gccctggagt agacaccaga agcccaagca tgggcagagg aaggcagggg ttggggggag 660
cagagctgtc tgtgttccag aagcccaagg acacagatgg ctaaggcgcc tgggagggac 720
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<210> 35
<211> 3633
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 35
tctagaatat agaagccaag gatttcaagg gtttcctttt ctctcttctt ctttttttct 60
ctttttcttt ttcctgagat ggagtttcct tttgtagccc tgactgtcct ggaattcact 120
ctatagacca ggctagcctc acacttagtg atctgcctgc ctctgcctct tgggtgcctc 180
aggattcaag gcatgaacca ccactacccg accagggatt tcttacacac ttctgactgg 240
actaaccagg aaagcagaga gggagacagg aagaaaatgc tcagaaggaa ggagtaggat 300
tggaggtgag ctgggggaac ccagactgag ccgtgcagaa gacaaggaag aagaaagcca 360
cccacacacc taggatccac ccacagattt tgctctgggt acccctgtct ggagactgta 420
gggctttgtg atggagggtg gggtagtctt catgccccgt gccctttact ccagacctaa 480
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cagttctcca tccctggtct gcagccaagg ctgggggcag aagaactttc tggaggattt 600
gagtgagaaa agcaagagag cctcaagtag ggactggaac ctctgggaag ggagtgcaga 660
ggagacccgg gtatgtgccc tacctggtac atttatacct gggcagcctc tgctcctgtt 720
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atttggagag agacagaact cagagctcag cactttcctc tttctgtttt tcttcttgag 840
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ggacggagca ggaggcacac ggagtgaggc cacgcatgag ccgaagctaa ccccccaccc 3600
cagccgcaaa gagtctacat gtctagggtc tag 3633

<210> 36
<211> 1404
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 36
tggtggtggt ggacaactag gaaactctgg cgctttctcc tcccctcaca aaactgagtc 60

cagctggagc cgcctccaga ctctctggcc agggcctcag agtggtcaac agtccctggc 120
cagcgttgct ctctccaggc taagggcacc cactcccctg gagattcctg aacctgggcc 180
aggaagagcc gaattagaca agtgtctcca atccggctgc gtgcggattt tgttgcggtg 240
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caggtccccc tctgtgtagg aatacaccag accctctctt agccacacac acctccagtc 360
ccccgtctac ctagattttt ttcatagcta gttggatggg ggatgggtta gggaggctgg 420
gtttgcgagc ctccaggtgg gagttcaccg acaggtactc cgcaaaggag ctggaaggca 480
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ggctcccccg cccctaaact ccccatcccc acctcctttc tcccgttact tcgtcctccc 600
tcgcctttcc agcctcgagt ctaaagctcc atgcttatgc ctctgcaaac aacccccctc 660
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gcttccctga cttgcgaccc ttctcttctt ccctccgtcc gagttaggcg ccaagtccta 1320
ggcgcgtagt gcacaggaga acactgatcc taatcctaat tctgctagtg aggagttctg 1380
tcgcagcatc ctcagtcaga gtcg 1404

<210> 37
<211> 12745
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 37
atgcggtttt ggcagtacat caatgggcgt ggatagcggt ttgactcacg gggatttcca 60
agtctccacc ccattgacgt caatgggagt ttgttttggc accaaaatca acgggacttt 120
ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg gcggtaggcg tgtacggtgg 180
gaggtctata taagcagagc tctgtgaaac ttcgaggagt ctctttgttg aggacttttg 240
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

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Phe Leu Phe His Ser Glu Thr Ile Cys Arg Pro Ser Gly Arg Lys Ser
20 25 30
Ser Lys Met Gln Ala Phe Arg Ile Trp Asp Val Asn Gln Lys Thr Phe
35 40 45
Tyr Leu Arg Asn Asn Gln Leu Val Ala Gly Tyr Leu Gln Gly Pro Asn
50 55 60
Val Asn Leu Glu Glu Lys Ile Asp Val Val Pro Ile Glu Pro His Ala
65 70 75 80
Leu Phe Leu Gly Ile His Gly Gly Lys Met Cys Leu Ser Cys Val Lys
85 90 95
Ser Gly Asp Glu Thr Arg Leu Gln Leu Glu Ala Val Asn Ile Thr Asp
100 105 110
Leu Ser Glu Asn Arg Lys Gln Asp Lys Arg Phe Ala Phe Ile Arg Ser
115 120 125
Asp Ser Gly Pro Thr Thr Ser Phe Glu Ser Ala Ala Cys Pro Gly Trp
130 135 140
Phe Leu Cys Thr Ala Met Glu Ala Asp Gln Pro Val Ser Leu Thr Asn
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Met Pro Asp Glu Gly Val Met Val Thr Lys Phe Tyr Phe Gln Glu Asp
165 170 175
Glu

<210> 39
<211> 569
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

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Ser Leu Glu Ala Asp Lys Cys Lys Glu Arg Glu Glu Lys Ile Ile Leu
20 25 30
Val Ser Ser Ala Asn Glu Ile Asp Val Arg Pro Cys Pro Leu Asn Pro
35 40 45
Asn Glu His Lys Gly Thr Ile Thr Trp Tyr Lys Asp Asp Ser Lys Thr
50 55 60
Pro Val Ser Thr Glu Gln Ala Ser Arg Ile His Gln His Lys Glu Lys

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Leu Trp Phe Val Pro Ala Lys Val Glu Asp Ser Gly His Tyr Tyr Cys
                85                  90                  95
Val Val Arg Asn Ser Ser Tyr Cys Leu Arg Ile Lys Ile Ser Ala Lys
            100                 105                 110
Phe Val Glu Asn Glu Pro Asn Leu Cys Tyr Asn Ala Gln Ala Ile Phe
        115                 120                 125
Lys Gln Lys Leu Pro Val Ala Gly Asp Gly Gly Leu Val Cys Pro Tyr
    130                 135                 140
Met Glu Phe Phe Lys Asn Glu Asn Asn Glu Leu Pro Lys Leu Gln Trp
145                 150                 155                 160
Tyr Lys Asp Cys Lys Pro Leu Leu Leu Asp Asn Ile His Phe Ser Gly
                165                 170                 175
Val Lys Asp Arg Leu Ile Val Met Asn Val Ala Glu Lys His Arg Gly
            180                 185                 190
Asn Tyr Thr Cys His Ala Ser Tyr Thr Tyr Leu Gly Lys Gln Tyr Pro
        195                 200                 205
Ile Thr Arg Val Ile Glu Phe Ile Thr Leu Glu Glu Asn Lys Pro Thr
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Arg Pro Val Ile Val Ser Pro Ala Asn Glu Thr Met Glu Val Asp Leu
225                 230                 235                 240
Gly Ser Gln Ile Gln Leu Ile Cys Asn Val Thr Gly Gln Leu Ser Asp
                245                 250                 255
Ile Ala Tyr Trp Lys Trp Asn Gly Ser Val Ile Asp Glu Asp Asp Pro
            260                 265                 270
Val Leu Gly Glu Asp Tyr Tyr Ser Val Glu Asn Pro Ala Asn Lys Arg
        275                 280                 285
Arg Ser Thr Leu Ile Thr Val Leu Asn Ile Ser Glu Ile Glu Ser Arg
    290                 295                 300
Phe Tyr Lys His Pro Phe Thr Cys Phe Ala Lys Asn Thr His Gly Ile
305                 310                 315                 320
Asp Ala Ala Tyr Ile Gln Leu Ile Tyr Pro Val Thr Asn Phe Gln Lys
                325                 330                 335
His Met Ile Gly Ile Cys Val Thr Leu Thr Val Ile Ile Val Cys Ser
            340                 345                 350
Val Phe Ile Tyr Lys Ile Phe Lys Ile Asp Ile Val Leu Trp Tyr Arg
        355                 360                 365
Asp Ser Cys Tyr Asp Phe Leu Pro Ile Lys Ala Ser Asp Gly Lys Thr
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Tyr Asp Ala Tyr Ile Leu Tyr Pro Lys Thr Val Gly Glu Gly Ser Thr
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Ser Asp Cys Asp Ile Phe Val Phe Lys Val Leu Pro Glu Val Leu Glu
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Lys Gln Cys Gly Tyr Lys Leu Phe Ile Tyr Gly Arg Asp Asp Tyr Val
            420                 425                 430
Gly Glu Asp Ile Val Glu Val Ile Asn Glu Asn Val Lys Lys Ser Arg
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Arg Leu Ile Ile Ile Leu Val Arg Glu Thr Ser Gly Phe Ser Trp Leu
    450                 455                 460
Gly Gly Ser Ser Glu Glu Gln Ile Ala Met Tyr Asn Ala Leu Val Gln
465                 470                 475                 480
Asp Gly Ile Lys Val Val Leu Leu Glu Leu Glu Lys Ile Gln Asp Tyr
                485                 490                 495
Glu Lys Met Pro Glu Ser Ile Lys Phe Ile Lys Gln Lys His Gly Ala
            500                 505                 510
Ile Arg Trp Ser Gly Asp Phe Thr Gln Gly Pro Gln Ser Ala Lys Thr
        515                 520                 525
Arg Phe Trp Lys Asn Val Arg Tyr His Met Pro Val Gln Arg Arg Ser
    530                 535                 540
Pro Ser Ser Lys His Gln Leu Leu Ser Pro Ala Thr Lys Glu Lys Leu
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Gln Arg Glu Ala His Val Pro Leu Gly
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<210> 40
<211> 398
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 40
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Gln Pro Ala Ala His Thr Gly Ala Ala Arg Ser Cys Arg Phe Arg Gly
20 25 30
Arg His Tyr Lys Arg Glu Phe Arg Leu Glu Gly Glu Pro Val Ala Leu
35 40 45
Arg Cys Pro Gln Val Pro Tyr Trp Leu Trp Ala Ser Val Ser Pro Arg
50 55 60
Ile Asn Leu Thr Trp His Lys Asn Asp Ser Ala Arg Thr Val Pro Gly
65 70 75 80
Glu Glu Glu Thr Arg Met Trp Ala Gln Asp Gly Ala Leu Trp Leu Leu
85 90 95
Pro Ala Leu Gln Glu Asp Ser Gly Thr Tyr Val Cys Thr Thr Arg Asn
100 105 110
Ala Ser Tyr Cys Asp Lys Met Ser Ile Glu Leu Arg Val Phe Glu Asn
115 120 125
Thr Asp Ala Phe Leu Pro Phe Ile Ser Tyr Pro Gln Ile Leu Thr Leu
130 135 140
Ser Thr Ser Gly Val Leu Val Cys Pro Asp Leu Ser Glu Phe Thr Arg
145 150 155 160
Asp Lys Thr Asp Val Lys Ile Gln Trp Tyr Lys Asp Ser Leu Leu Leu
165 170 175
Asp Lys Asp Asn Glu Lys Phe Leu Ser Val Arg Gly Thr Thr His Leu
180 185 190
Leu Val His Asp Val Ala Leu Glu Asp Ala Gly Tyr Tyr Arg Cys Val
195 200 205
Leu Thr Phe Ala His Glu Gly Gln Gln Tyr Asn Ile Thr Arg Ser Ile
210 215 220
Glu Leu Arg Ile Lys Lys Lys Lys Glu Glu Thr Ile Pro Val Ile Ile
225 230 235 240
Ser Pro Leu Lys Thr Ile Ser Ala Ser Leu Gly Ser Arg Leu Thr Ile
245 250 255
Pro Cys Lys Val Phe Leu Gly Thr Gly Thr Pro Leu Thr Thr Met Leu
260 265 270
Trp Trp Thr Ala Asn Asp Thr His Ile Glu Ser Ala Tyr Pro Gly Gly
275 280 285
Arg Val Thr Glu Gly Pro Arg Gln Glu Tyr Ser Glu Asn Asn Glu Asn
290 295 300
Tyr Ile Glu Val Pro Leu Ile Phe Asp Pro Val Thr Arg Glu Asp Leu
305 310 315 320
His Met Asp Phe Lys Cys Val Val His Asn Thr Leu Ser Phe Gln Thr
325 330 335
Leu Arg Thr Thr Val Lys Glu Ala Ser Ser Thr Phe Ser Trp Gly Ile
340 345 350
Val Leu Ala Pro Leu Ser Leu Ala Phe Leu Val Leu Gly Gly Ile Trp
355 360 365
Met His Arg Arg Cys Lys His Arg Thr Gly Lys Ala Asp Gly Leu Thr
370 375 380

Val Leu Trp Pro His His Gln Asp Phe Gln Ser Tyr Pro Lys
385 390 395

<210> 41
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 41
ggaagcgaua auuuuaagct t 21

<210> 42
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 42
ggagaauccg gccagucaut t 21

<210> 43
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 43
ggguugauua uguaccauut t 21

<210> 44
<211> 21

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 44
ggcuucacua aggguugaut t 21

<210> 45
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 45
ggcaguaucc uuaugcaugt t 21

<210> 46
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 46
gcuuuuacau cucuuagcat t 21

<210> 47
<211> 21
<212> DNA
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<220>
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synthetic construct

<400> 47
gggaagaaac aguuaccagt t 21

<210> 48
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<212> DNA
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<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 48
gggcaccaac auccuguuut t 21

<210> 49
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<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 49
ggaugggaaa cuuaaguact t 21

<210> 50
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 50
ccuacaacuc agcgcaugat t 21

<210> 51
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 51
gcgcaugacu acaucagcut t 21

<210> 52
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 52
gcuacgagca guuuuuauut t 21

<210> 53
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 53
ggauuugacc aguauaagut t 21

<210> 54
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 54
gggagucugg aacauugugt t 21

<210> 55
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 55

gguuuuuagu aucagaacut t 21

<210> 56
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 56
gcacaggauu ugaccaguat t 21

<210> 57
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 57
gggaaauaag gagcuuccut t 21

<210> 58
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 58
cccuacagua cuaaucaaat t 21

<210> 59
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 59
ggcuuuugcc aaccccgagt t 21

<210> 60
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 60

gatccggggt tgattatgta ccattttcaa gagaaatggt acataatcaa ccctttttg 59

<210> 61
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 61
gccccaacta atacatggta aaagttctct ttaccatgta ttagttggga aaaacttaa 59

<210> 62
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 62
gatccgggct tttgccaacc ccgagttcaa gagactcggg gttggcaaaa gcctttttg 59

<210> 63
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 63
gcccgaaaac ggttggggct caagttctct gagccccaac cgttttcgga aaaacttaa 59

<210> 64
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 64
gatccgggat gggaaactta agtacttcaa gagagtactt aagtttccca tcctttttg 59

<210> 65
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 65
gatccgggat gggaaactta agtacttcaa gagagtactt aagtttccca tcctttttg 59

<210> 66
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 66
gatccgggat ttgaccagta taagtttcaa gagaacttat actggtcaaa tccttttg 59

<210> 67
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 67
gccctaaact ggtcatattc aaagttctct tgaatatgac cagtttagga aaaacttaa 59

<210> 68
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 68
Met Glu Ile Cys Arg Gly Leu Arg Ser His Leu Ile Thr Leu Leu Leu
1 5 10 15
Phe Leu Phe His Ser Glu Thr Ile Cys
20 25

<210> 69
<211> 1774
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 69
ggttcaagtg attctgctgc ctcagcctcc caggcgggat tacaggtgcc tgccaccacg 60
cctggctaat ttttttgtct ttttagtaaa gatgaggttt caccatgttg ggcaggctgg 120
tttcaattgc tgacctcaag tgagccaccc cgcctcagcc tccaaaatgc taggattaca 180
ggcatgagcc accgcaccca gccaagtttg tacatatatt tttgactaca cttcttaact 240
attcttagga taaattacta gaagtgaaaa ttcttgggtg aagagcttga ggcctttaca 300
cacacacaca cacacacaca cacacacaca caaataggct ggatcgagtg gctcacacct 360
gtaatctcag cagtttggga ggctgaggaa ggaggatcac ttgagtccag gaggttgaga 420
atagcctgaa caacatagca agatcttgtc tctacaaaaa agtttaaaaa aaattagctg 480
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tgagcccagg aggtgattga agctgcagtg agctgtgatt acaccactgc actccagcct 600
gggcaacaga gctagactct gtctctaaaa aaaggcacaa aataatattt aaaaagcacc 660

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ggtaactttt gggtctgtca taaatagagg gccc 1774

<210> 70
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 70
gttccctttc tgccagccct 20

<210> 71
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 71
gtgccgtgag tttcccaga 19

<210> 72
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note =
synthetic construct

<400> 72
cccacttcct tgccctctca 20

<210> 73
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note = synthetic construct

<400> 73
actctgttgt gttcccgca 19

<210> 74
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note = synthetic construct

<400> 74
cctcttctcg cttccctca 19

<210> 75
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note = synthetic construct

<400> 75
gttcccatca gccacttcgt 20

<210> 76
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note = synthetic construct

<400> 76
atcctttgat cugaugaguc cgugaggacg aaagttataa gcac 44

<210> 77
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note = synthetic construct

<400> 77
tctttagaac ugaugagucc gugaggacga aagacaaatt gca 43

<210> 78
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
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<400> 78
ggccgaagcg cugaugaguc cgugaggacg aaagacagat gccc 44

<210> 79
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
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<400> 79
tggatgtcaa cugaugaguc cgugaggacg aaagataact cata 44

<210> 80
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note = synthetic construct

<400> 80
aggagttcga cugaugaguc cgugaggacg aaagcctcct gggc 44

<210> 81
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note = synthetic construct

<400> 81
tctccggtgc cugaugaguc cgugaggacg aaagtccgct tttt 44

<210> 82
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequences; note = synthetic construct

<400> 82
cugaugaguc cgugaggacg aaag 24